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(57) Abstract

A recombinant adenovirus and a method for producing the virus are provided which utilize a recombinant shuttle vector comprising adenovirus DNA sequence for the 5' and 3' cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes and a selected minigene linked thereto, and a helper adenovirus comprising sufficient adenovirus gene sequences necessary for a productive viral infection. Desirably the helper gene is crippled by modifications to its 5' packaging sequences, which facilitates purification of the viral particle from the helper virus.

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IMPROVED ADENOVIRUS AND METHODS OF USE THEREOF

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Field of the Invention

The present invention relates to the field of vectors useful in somatic gene therapy and the production thereof.

Background of the Invention

Human gene therapy is an approach to treating human disease that is based on the modification of gene expression in cells of the patient. It has become apparent over the last decade that the single most outstanding barrier to the success of gene therapy as a strategy for treating inherited diseases, cancer, and other genetic dysfunctions is the development of useful gene transfer vehicles. Eukaryotic viruses have been employed as vehicles for somatic gene therapy. Among the viral vectors that have been cited frequently in gene therapy research are adenoviruses.

Adenoviruses are eukaryotic DNA viruses that can be modified to efficiently deliver a therapeutic or reporter transgene to a variety of cell types. Recombinant adenoviruses types 2 and 5 (Ad2 and Ad5, respectively), which cause respiratory disease in humans, are currently being developed for gene therapy. Both Ad2 and Ad5 belong to a subclass of adenovirus that are not associated with human malignancies. Recombinant adenoviruses are capable of providing extremely high levels of transgene delivery to virtually all cell types, regardless of the mitotic state. High titers (10^{13} plaque forming units/ml) of recombinant virus can be easily generated in 293 cells (the adenovirus equivalent

to retrovirus packaging cell lines) and cryo-stored for extended periods without appreciable losses. The efficacy of this system in delivering a therapeutic transgene *in vivo* that complements a genetic imbalance has been demonstrated in anima. models of various disorders [Y. Watanabe, Atherosclerosis, 36:261-268 (1986); K. Tanzawa et al, FEBS Letters, 118(1):81-84 (1980); J.L. Golasten et al, New Engl. J. Med., 309(11983):288-296 (1983); S. Ishibashi et al, J. Clin. Invest., 92:883-893 (1993); and S. Ishibashi et al, J. Clin. Invest., 93:1885-1893 (1994)]. Indeed, a recombinant replication defective adenovirus encoding a cDNA for the cystic fibrosis transmembrane regulator (CFTR) has been approved for use in at least two human CF clinical trials [see, e.g., J. Wilson, Nature, 365:691-692 (Oct. 21, 1993)]. Further support of the safety of recombinant adenoviruses for gene therapy is the extensive experience of live adenovirus vaccines in human populations.

Human adenoviruses are comprised of a linear, approximately 36 kb double-stranded DNA genome, which is divided into 100 map units (m.u.), each of which is 360 bp in length. The DNA contains short inverted terminal repeats (ITR) at each end of the genome that are required for viral DNA replication. The gene products are organized into early (E1 through E4) and late (L1 through L5) regions, based on expression before or after the initiation of viral DNA synthesis [see, e.g., Horwitz, Virology, 2d edit., ed. B. N. Fields, Raven Press, Ltd. , New York (1990)].

The first-generation recombinant, replication-deficient adenoviruses which have been developed for gene therapy contain deletions of the entire E1a and part of the E1b regions. This replication-defective virus is grown on an adenovirus-transformed, complementation human

embryonic kidney cell line containing a functional adenovirus E1a gene which provides a transacting E1a protein, the 293 cell [ATCC CRL1573]. E1-deleted viruses are capable of replicating and producing infectious virus in the 293 cells, which provide E1a and E1b region gene products in *trans*. The resulting virus is capable of infecting many cell types and can express the introduced gene (providing it carries its own promoter), but cannot replicate in a cell that does not carry the E1 region DNA unless the cell is infected at a very high multiplicity of infection.

However, *in vivo* studies revealed transgene expression in these E1 deleted vectors was transient and invariably associated with the development of severe inflammation at the site of vector targeting [S. Ishibashi et al, J. Clin. Invest., 93:1885-1893 (1994); J. M. Wilson et al, Proc. Natl. Acad. Sci., USA, 85:4421-4424 (1988); J. M. Wilson et al, Clin. Bio., 3:21-26 (1991); M. Grossman et al, Som. Cell. and Mol. Gen., 17:601-607 (1991)]. One explanation that has been proposed to explain this finding is that first generation recombinant adenoviruses, despite the deletion of E1 genes, express low levels of other viral proteins. This could be due to basal expression from the unstimulated viral promoters or transactivation by cellular factors. Expression of viral proteins leads to cellular immune responses to the genetically modified cells, resulting in their destruction and replacement with nontransgene containing cells.

There yet remains a need in the art for the development of additional adenovirus vector constructs for gene therapy.

Summary of the Invention

In one aspect, the invention provides the components of a novel recombinant adenovirus production system. One component is a shuttle plasmid, pAdΔ, that comprises adenovirus cis-elements necessary for replication and virion encapsidation and is deleted of all viral genes. This vector carries a selected transgene under the control of a selected promoter and other conventional vector/plasmid regulatory components. The other component is a helper adenovirus, which alone or with a packaging cell line, supplies sufficient gene sequences necessary for a productive viral infection. In a preferred embodiment, the helper virus has been altered to contain modifications to the native gene sequences which direct efficient packaging, so as to substantially disable or "cripple" the packaging function of the helper virus or its ability to replicate.

In another aspect, the present invention provides a unique recombinant adenovirus, an AdΔ virus, produced by use of the components above. This recombinant virus comprises an adenovirus capsid, adenovirus cis-elements necessary for replication and virion encapsidation, but is deleted of all viral genes (i.e., all viral open reading frames). This virus particle carries a selected transgene under the control of a selected promoter and other conventional vector regulatory components. This AdΔ recombinant virus is characterized by high titer transgene delivery to a host cell and the ability to stably integrate the transgene into the host cell chromosome. In one embodiment, the virus carries as its transgene a reporter gene. Another embodiment of the recombinant virus contains a therapeutic transgene.

In another aspect, the invention provides a method for producing the above-described recombinant AdΔ virus by co-transfecting a cell line (either a packaging cell

line or a non-packaging cell line) with a shuttle vector or plasmid and a helper adenovirus as described above, wherein the transfected cell generates the AdΔ virus. The AdΔ virus is subsequently isolated and purified therefrom.

In yet a further aspect, the invention provides a method for delivering a selected gene to a host cell for expression in that cell by administering an effective amount of a recombinant AdΔ virus containing a therapeutic transgene to a patient to treat or correct a genetically associated disorder or disease.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

Brief Description of the Figures

Fig. 1A is a schematic representation of the organization of the major functional elements that define the 5' terminus from Ad5 including an inverted terminal repeat (ITR) and a packaging/enhancer domain. The TATA box of the E1 promoter (black box) and E1A transcriptional start site (arrow) are also shown.

Fig. 1B is an expanded schematic of the packaging/enhancer region of Fig. 1A, indicating the five packaging (PAC) domains (A-repeats), I through V. The arrows indicate the location of PCR primers referenced in Figs. 9A and 9B below.

Fig. 2A is a schematic of shuttle vector pAdΔ.CMVLacZ containing 5' ITR from Ad5, followed by a CMV promoter/enhancer, a LacZ gene, a 3' ITR from Ad5, and remaining plasmid sequence from plasmid pSP72 backbone. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Fig. 2B is a schematic of the shuttle vector digested with EcoRI to release the modified AdΔ genome from the pSP72 plasmid backbone.

Fig. 2C is a schematic depiction of the function of the vector system. In the presence of an E1-deleted helper virus Ad.CBhpAP which encodes a reporter minigene for human placenta alkaline phosphatase (hpAP), the AdΔ.CMVLacZ genome is packaged into preformed virion capsids, distinguishable from the helper virions by the presence of the LacZ gene.

Figs. 3A to 3F [SEQ ID NO: 1] report the top DNA strand of the double-stranded plasmid pAdΔ.CMVLacZ. The complementary sequence may be readily obtained by one of skill in the art. The sequence includes the following components: 3' Ad ITR (nucleotides 607-28 of SEQ ID NO: 1); the 5' Ad ITR (nucleotides 5496-5144 of SEQ ID NO: 1); CMV promoter/enhancer (nucleotides 5117-4524 of SEQ ID NO: 1); SD/SA sequence (nucleotides 4507-4376 of SEQ ID NO: 1); LacZ gene (nucleotides 4320-845 of SEQ ID NO: 1); and a poly A sequence (nucleotides 837-639 of SEQ ID NO: 1).

Fig. 4A is a schematic of shuttle vector pAdΔc.CMVLacZ containing an Ad5 5' ITR and 3' ITR positioned head-to-tail, with a CMV enhancer/promoter-LacZ minigene immediately following the 5' ITR, followed by a plasmid pSP72 (Promega) backbone. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Fig. 4B is a schematic depiction of the function of the vector system of Fig. 4A. In the presence of helper virus Ad.CBhpAP, the circular pADΔc.CMVLacZ shuttle vector sequence is packaged into virion heads, distinguishable from the helper virions by the presence of the LacZ gene.

Figs. 5A to 5F [SEQ ID NO: 2] report the top DNA strand of the double-stranded vector pAdΔc.CMVLacZ. The complementary sequence may be readily obtained by one of skill in the art. The sequence includes the following components: 5' Ad ITR (nucleotides 600-958 of SEQ ID NO: 2); CMV promoter/enhancer (nucleotides 969-1563 of SEQ ID NO: 2); SD/SA sequence (nucleotides 1579-1711); LacZ gene (nucleotides 1762-5236 of SEQ ID NO: 2); poly A sequence (nucleotides 5245-5443 of SEQ ID NO: 2); and 3' Ad ITR (nucleotides 16-596 of SEQ ID NO: 2).

Fig. 6 is a schematic of shuttle vector pAdΔ.CBCFTR containing 5' ITR from Ad5, followed by a chimeric CMV enhancer/β actin promoter enhancer, a CFTR gene, a poly-A sequence, a 3' ITR from Ad5, and remaining plasmid sequence from plasmid pSL1180 (Pharmacia) backbone. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Figs. 7A to 7H [SEQ ID NO: 3] report the top DNA strand of the double-stranded plasmid pAdΔ.CBCFTR. The complementary sequence may be readily obtained by one of skill in the art. The sequence includes the following components: 5' Ad ITR (nucleotides 9611-9254 of SEQ ID NO: 3); chimeric CMV enhancer/β actin promoter (nucleotides 9241-8684 of SEQ ID NO: 3); CFTR gene (nucleotides 8622-4065 of SEQ ID NO: 3); poly A sequence (nucleotides 3887-3684 of SEQ ID NO: 3); and 3' Ad ITR (nucleotides 3652-3073 of SEQ ID NO: 3). The remaining plasmid backbone is obtained from pSL1180 (Pharmacia).

Fig. 8A illustrates the generation of 5' adenovirus terminal sequence that contained PAC domains I and II by PCR. See, arrows indicating righthand and lefthand (PAC II) PCR probes in Fig. 1B.

Fig. 8B illustrates the generation of 5' terminal sequence that contained PAC domains I, II, III and IV by PCR. See, arrows indicating righthand and lefthand (PAC IV) PCR probes in Fig. 1B.

Fig. 8C depicts the amplification products subcloned into the multiple cloning site of pAd.Link.1 (IHGT Vector Core) generating pAd.PACII (domains I and II) and pAd.PACIV (domains I, II, III, and IV) resulting in crippled helper viruses, Ad.PACII and Ad.PACIV with modified packaging (PAC) signals.

Fig. 9A is a schematic representation of the subcloning of a human placenta alkaline phosphatase reporter minigene containing the immediate early CMV enhancer/ promoter (CMV), human placenta alkaline phosphatase cDNA (hpAP), and SV40 polyadenylation signal (pA) into pAd.PACII to result in crippled helper virus vector pAdΔ.PACII.CMVhpAP. Restriction endonuclease enzymes are represented by conventional designations in the plasmid constructs.

Fig. 9B is a schematic representation of the subcloning of the same minigene of Fig. 9A into pAd.PACIV to result in crippled helper virus vector pAd.PACIV.CMV.hpAP.

Fig. 10 is a flow diagram summarizing the synthesis of an adenovirus-based polycation helper virus conjugate and its combination with a pAdΔ shuttle vector to result in a novel viral particle complex. CsCl band purified helper adenovirus was reacted with the heterobifunctional crosslinker sulfo-SMCC and the capsid protein fiber is labeled with the nucleophilic maleimide moiety. Free sulfhydryls were introduced onto poly-L-lysine using 2-iminothiolane-HCl and mixed with the labelled adenovirus, resulting in the helper virus conjugate Ad-pLys. A unique adenovirus-based particle is generated by purifying the Ad-pLys conjugate over a CsCl gradient to

remove unincorporated poly-L-lysine, followed by extensively dialyzing, adding shuttle plasmid DNAs to Ad-pLys and allowing the complex formed by the shuttle plasmid wrapped around Ad-pLys to develop.

Fig. 11 is a schematic diagram of pCCL-DMD, which is described in detail in Example 9 below.

Fig. 12A - 12P provides the continuous DNA sequence of pAdΔ.CMVmDys [SEQ ID NO:10].

Detailed Description of the Invention

The present invention provides a unique recombinant adenovirus capable of delivering transgenes to target cells, as well as the components for production of the unique virus and methods for the use of the virus to treat a variety of genetic disorders.

The AdΔ virus of this invention is a viral particle containing only the adenovirus cis-elements necessary for replication and virion encapsidation (i.e., ITRs and packaging sequences), but otherwise deleted of all adenovirus genes (i.e., all viral open reading frames). This virus carries a selected transgene under the control of a selected promoter and other conventional regulatory components, such as a poly A signal. The AdΔ virus is characterized by improved persistence of the vector DNA in the host cells, reduced antigenicity/immunogenicity, and hence, improved performance as a delivery vehicle. An additional advantage of this invention is that the AdΔ virus permits the packaging of very large transgenes, such as a full-length dystrophin cDNA for the treatment of the progressive wasting of muscle tissue characteristic of Duchenne Muscular Dystrophy (DMD).

This novel recombinant virus is produced by use of an adenovirus-based vector production system containing two components: 1) a shuttle vector that comprises adenovirus cis-elements necessary for replication and

virion encapsidation and is deleted of all viral genes, which vector carries a reporter or therapeutic minigene and 2) a helper adenovirus which, alone or with a packaging cell line, is capable of providing all of the viral gene products necessary for a productive viral infection when co-transfected with the shuttle vector. Preferably, the helper virus is modified so that it does not package itself efficiently. In this setting, it is desirably used in combination with a packaging cell line that stably expresses adenovirus genes. The methods of producing this viral vector from these components include both a novel means of packaging of an adenoviral/transgene containing vector into a virus, and a novel method for the subsequent separation of the helper virus from the newly formed recombinant virus.

I. The Shuttle Vector

The shuttle vector, referred to as pAdΔ, is composed of adenovirus sequences, and transgene sequences, including vector regulatory control sequences.

A. *The Adenovirus Sequences*

The adenovirus nucleic acid sequences of the shuttle vector provide the minimum adenovirus sequences which enable a viral particle to be produced with the assistance of a helper virus. These sequences assist in delivery of a recombinant transgene genome to a target cell by the resulting recombinant virus.

The DNA sequences of a number of adenovirus types are available from Genbank, including type Ad5 [Genbank Accession No. M73260]. The adenovirus sequences may be obtained from any known adenovirus serotype, such as serotypes 2, 3, 4, 7, 12 and 40, and further including any of the presently identified 41 human types [see, e.g., Horwitz, cited above]. Similarly adenoviruses known to infect other animals may also be employed in the

vector constructs of this invention. The selection of the adenovirus type is not anticipated to limit the following invention. A variety of adenovirus strains are available from the American Type Culture Collection, Rockville, Maryland, or available by request from a variety of commercial and institutional sources. In the following exemplary embodiment an adenovirus, type 5 (Ad5) is used for convenience.

However, it is desirable to obtain a variety of pAdΔ shuttle vectors based on different human adenovirus serotypes. It is anticipated that a library of such plasmids and the resulting AdΔ viral vectors would be useful in a therapeutic regimen to evade cellular, and possibly humoral, immunity, and lengthen the duration of transgene expression, as well as improve the success of repeat therapeutic treatments. Additionally the use of various serotypes is believed to produce recombinant viruses with different tissue targeting specificities. The absence of adenoviral genes in the AdΔ viral vector is anticipated to reduce or eliminate adverse CTL response which normally causes destruction of recombinant adenoviruses deleted of only the E1 gene.

Specifically, the adenovirus nucleic acid sequences employed in the pAdΔ shuttle vector of this invention are adenovirus genomic sequences from which all viral genes are deleted. More specifically, the adenovirus sequences employed are the cis-acting 5' and 3' inverted terminal repeat (ITR) sequences of an adenovirus (which function as origins of replication) and the native 5' packaging/enhancer domain, that contains sequences necessary for packaging linear Ad genomes and enhancer elements for the E1 promoter. These sequences are the sequences necessary for replication and virion encapsidation. See, e.g., P. Hearing et al, J. Virol., 61(8):2555-2558 (1987); M. Grable and P. Hearing, J.

Virol., 64(5): 2047-2056 (1990); and M. Grable and P. Hearing, J. Virol., 66(2):723-731 (1992).

According to this invention, the entire adenovirus 5' sequence containing the 5' ITR and packaging/enhancer region can be employed as the 5' adenovirus sequence in the pAdΔ shuttle vector. This left terminal (5') sequence of the Ad5 genome useful in this invention spans bp 1 to about 360 of the conventional adenovirus genome, also referred to as map units 0-1 of the viral genome. This sequence is provided herein as nucleotides 5496-5144 of SEQ ID NO: 1, nucleotides 600-958 of SEQ ID NO: 2; and nucleotides 9611-9254 of SEQ ID NO: 3, and generally is from about 353 to about 360 nucleotides in length. This sequence includes the 5' ITR (bp 1-103 of the adenovirus genome), and the packaging/enhancer domain (bp 194-358 of the adenovirus genome). See, Figs. 1A, 3, 5, and 7.

Preferably, this native adenovirus 5' region is employed in the shuttle vector in unmodified form. However, some modifications including deletions, substitutions and additions to this sequence which do not adversely effect its biological function may be acceptable. See, e.g., WO 93/24641, published December 9, 1993. The ability to modify these ITR sequences is within the ability of one of skill in the art. See, e.g., texts such as Sambrook et al, "Molecular Cloning. A Laboratory Manual.", 2d edit., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989).

The 3' adenovirus sequences of the shuttle vector include the right terminal (3') ITR sequence of the adenoviral genome spanning about bp 35,353 - end of the adenovirus genome, or map units ~98.4-100. This sequence is provided herein as nucleotides 607-28 of SEQ ID NO: 1, nucleotides 16-596 of SEQ ID NO: 2; and nucleotides 3652-3073 of SEQ ID NO: 3, and generally is

about 580 nucleotides in length. This entire sequence is desirably employed as the 3' sequence of an pAdΔ shuttle vector. Preferably, the native adenovirus 3' region is employed in the shuttle vector in unmodified form. However, some modifications to this sequence which do not adversely effect its biological function may be acceptable.

An exemplary pAdΔ shuttle vector of this invention, described below and in Fig. 2A, contains only those adenovirus sequences required for packaging adenoviral genomic DNA into a preformed capsid head. The pAdΔ vector contains Ad5 sequences encoding the 5' terminal and 3' terminal sequences (identified in the description of Fig. 3), as well as the transgene sequences described below.

From the foregoing information, it is expected that one of skill in the art may employ other equivalent adenovirus sequences for use in the AdΔ vectors of this invention. These sequences may include other adenovirus strains, or the above mentioned cis-acting sequences with minor modifications.

B. The Transgene

The transgene sequence of the vector and recombinant virus is a nucleic acid sequence or reverse transcript thereof, heterologous to the adenovirus sequence, which encodes a polypeptide or protein of interest. The transgene is operatively linked to regulatory components in a manner which permits transgene transcription.

The composition of the transgene sequence will depend upon the use to which the resulting virus will be put. For example, one type of transgene sequence includes a reporter sequence, which upon expression produces a detectable signal. Such reporter sequences include without limitation an *E. coli* beta-galactosidase

(*LacZ*) cDNA, a human placental alkaline phosphatase gene and a green fluorescent protein gene. These sequences, when associated with regulatory elements which drive their expression, provide signals detectable by conventional means, e.g., ultraviolet wavelength absorbance, visible color change, etc.

Another type of transgene sequence includes a therapeutic gene which expresses a desired gene product in a host cell. These therapeutic nucleic acid sequences typically encode products for administration and expression in a patient *in vivo* or *ex vivo* to replace or correct an inherited or non-inherited genetic defect or treat an epigenetic disorder or disease. Such therapeutic genes which are desirable for the performance of gene therapy include, without limitation, a normal cystic fibrosis transmembrane regulator (CFTR) gene (see Fig. 7), a low density lipoprotein (LDL) gene [T. Yamamoto et al, Cell, 39:27-28 (November, 1984)], a DMD cDNA sequence [partial sequences available from GenBank, Accession Nos. M36673, M36671, [A. P. Monaco et al, Nature, 323:646-650 (1986)] and L06900, [Roberts et al, Hum. Mutat., 2:293-299 (1993)]] (Genbank), and a number of genes which may be readily selected by one of skill in the art. The selection of the transgene is not considered to be a limitation of this invention, as such selection is within the knowledge of the art-skilled.

C. Regulatory Elements

In addition to the major elements identified above for the pAdΔ shuttle vector, i.e., the adenovirus sequences and the transgene, the vector also includes conventional regulatory elements necessary to drive expression of the transgene in a cell transfected with the pAdΔ vector. Thus the vector contains a selected promoter which is linked to the transgene and located,

with the transgene, between the adenovirus sequences of the vector.

Selection of the promoter is a routine matter and is not a limitation of the pAdΔ vector itself. Useful promoters may be constitutive promoters or regulated (inducible) promoters, which will enable control of the amount of the transgene to be expressed. For example, a desirable promoter is that of the cytomegalovirus immediate early promoter/enhancer [see, e.g., Boshart et al, Cell, 41:521-530 (1985)]. This promoter is found at nucleotides 5117-4524 of SEQ ID NO: 1 and nucleotides 969-1563 of SEQ ID NO: 2. Another promoter is the CMV enhancer/chicken β-actin promoter (nucleotides 9241-8684 of SEQ ID NO: 3). Another desirable promoter includes, without limitation, the Rous sarcoma virus LTR promoter/enhancer. Still other promoter/enhancer sequences may be selected by one of skill in the art.

The shuttle vectors will also desirably contain nucleic acid sequences heterologous to the adenovirus sequences including sequences providing signals required for efficient polyadenylation of the transcript and introns with functional splice donor and acceptor sites (SD/SA). A common poly-A sequence which is employed in the exemplary vectors of this invention is that derived from the papovavirus SV-40 [see, e.g., nucleotides 837-639 of SEQ ID NO: 1; 5245-5443 of SEQ ID NO: 2; and 3887-3684 of SEQ ID NO: 3]. The poly-A sequence generally is inserted in the vector following the transgene sequences and before the 3' adenovirus sequences. A common intron sequence is also derived from SV-40, and is referred to as the SV-40 T intron sequence [see, e.g., nucleotides 4507-4376 of SEQ ID NO: 1 and 1579-1711 of SEQ ID NO: 2]. A pAdΔ shuttle vector of the present invention may also contain such an intron, desirably located between the

promoter/enhancer sequence and the transgene. Selection of these and other common vector elements are conventional and many such sequences are available [see, e.g., Sambrook et al, and references cited therein]. Examples of such regulatory sequences for the above are provided in the plasmid sequences of Figs. 3, 5 and 7.

The combination of the transgene, promoter/enhancer, the other regulatory vector elements are referred to as a "minigene" for ease of reference herein. The minigene is preferably flanked by the 5' and 3' cis-acting adenovirus sequences described above. Such a minigene may have a size in the range of several hundred base pairs up to about 30 kb due to the absence of adenovirus early and late gene sequences in the vector. Thus, this AdΔ vector system permits a great deal of latitude in the selection of the various components of the minigene, particularly the selected transgene, with regard to size. Provided with the teachings of this invention, the design of such a minigene can be made by resort to conventional techniques.

II. The Helper Virus

Because of the limited amount of adenovirus sequence present in the AdΔ shuttle vector, a helper adenovirus of this invention must, alone or in concert with a packaging cell line, provide sufficient adenovirus gene sequences necessary for a productive viral infection. Helper viruses useful in this invention thus contain selected adenovirus gene sequences, and optionally a second reporter minigene.

Normally, the production of a recombinant adenovirus which utilizes helper adenovirus containing a full complement of adenoviral genes results in recombinant virus contaminated by excess production of the helper virus. Thus, extensive purification of the viral vector

from the contaminating helper virus is required. However, the present invention provides a way to facilitate purification and reduce contamination by crippling the helper virus.

One preferred embodiment of a helper virus of this invention thus contains three components (A) modifications or deletions of the native adenoviral gene sequences which direct efficient packaging, so as to substantially disable or "cripple" the packaging function of the helper virus or its ability to replicate, (B) selected adenovirus genes and (C) an optional reporter minigene. These "crippled" helper viruses may also be formed into poly-cation conjugates as described below.

The adenovirus sequences forming the helper virus may be obtained from the sources identified above in the discussion of the shuttle vector. Use of different Ad serotypes as helper viruses enables production of recombinant viruses containing the ΔAd (serotype 5) shuttle vector sequences in a capsid formed by the other serotype adenovirus. These recombinant viruses are desirable in targeting different tissues, or evading an immune response to the ΔAd sequences having a serotype 5 capsid. Use of these different Ad serotype helper viruses may also demonstrate advantages in recombinant virus production, stability and better packaging.

A. *The Crippling Modifications*

A desirable helper virus used in the production of the adenovirus vector of this invention is modified (or crippled) in its 5' ITR packaging/enhancer domain, identified above. As stated above, the packaging/enhancer region contains sequences necessary for packaging linear adenovirus genomes ("PAC" sequences). More specifically, this sequence contains at least seven distinct yet functionally redundant domains

that are required for efficient encapsidation of replicated viral DNA.

Within a stretch of nucleotide sequence from bp 194-358 of the Ad5 genome, five of these so-called A-repeats or PAC sequences are localized (see, Fig. 1B). PAC I is located at bp 241-248 of the adenovirus genome (on the strand complementary to nucleotides 5259-5246 of SEQ ID NO: 1). PAC II is located at bp 262-269 of the adenovirus genome (on the strand complementary to nucleotides 5238-5225 of SEQ ID NO: 1). PAC III is located at bp 304-311 of the adenovirus genome (on the strand complementary to nucleotides 5196-5183 of SEQ ID NO: 1). PAC IV is located at bp 314-321 of the adenovirus (on the strand complementary to nucleotides 5186-5172 of SEQ ID NO: 1). PAC V is located at bp 339-346 of the adenovirus (on the strand complementary to nucleotides 5171-5147 of SEQ ID NO: 1).

Corresponding sequences can be obtained from SEQ ID NO: 2 and 3. PAC I is located at nucleotides 837-851 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9374-9360 of SEQ ID NO: 3. PAC II is located at nucleotides 859-863 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9353-9340 of SEQ ID NO: 3. PAC III is located at nucleotides 901-916 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9311-9298 of SEQ ID NO: 3. PAC IV is located at nucleotides 911-924 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9301-9288 of SEQ ID NO: 3. PAC V is located at nucleotides 936-949 of SEQ ID NO: 2; and on the strand complementary to nucleotides 9276-9263 of SEQ ID NO: 3.

Table 1 below lists these five native Ad5 sequences and a consensus PAC sequence based on the similarities between an eight nucleic acid stretch within the five sequences. The consensus sequence contains two positions at which the nucleic acid may be A or T (A/T). The conventional single letter designations are used for the nucleic acids, as is known to the art.

Table 1

A-Repeat	Adenovirus Genome Base Pair Nos. & Nucleotide sequence
	241 248
I	TAG TAAATTTG GGC [SEQ ID NO: 4]
	262 269
II	AGT AAGATTTG GCC [SEQ ID NO: 5]
	304 311
III	AGT GAAATCTG AAT [SEQ ID NO: 6]
	314 321
IV	GAA TAATTTTG TGT [SEQ ID NO: 7]
	339 346
V	CGT AATATTTG TCT [SEQ ID NO: 8]
Consensus	5' (A/T)AN(A/T)TTTG 3' [SEQ ID NO: 9]

According to this invention, mutations or deletions may be made to one or more of these PAC sequences to generate desirable crippled helper viruses. A deletion analysis of the packaging domain revealed a positive correlation between encapsidation efficiency and the number of packaging A-repeats that were present at the 5' end of the genome. Modifications of this domain may include 5' adenovirus sequences which contain less than all five of the PAC sequences of Table 1. For example, only two PAC sequences may be present in the crippled virus, e.g., PAC I and PAC II, PAC III and PAC IV, and so on. Deletions of selected PAC sequences may

involve deletion of contiguous or non-contiguous sequences. For example, PAC II and PAC IV may be deleted, leaving PAC I, III and IV in the 5' sequence. Still an alternative modification may be the replacement of one or more of the native PAC sequences with one or more repeats of the consensus sequence of Table 1. Alternatively, this adenovirus region may be modified by deliberately inserted mutations which disrupt one or more of the native PAC sequences. One of skill in the art may further manipulate the PAC sequences to similarly achieve the effect of reducing the helper virus packaging efficiency to a desired level.

Exemplary helper viruses which involve the manipulation of the PAC sequences described above are disclosed in Example 7 below. Briefly, as described in that example, one helper virus contains in place of the native 5' ITR region (adenovirus genome bp 1-360), a 5' adenovirus sequence spanning adenovirus genome bp 1-269, which contains only the 5' ITR and PAC I and PAC II sequences, and deletes the adenovirus region bp 270-360.

Another PAC sequence modified helper virus contains only the 5' Ad5 sequence of the ITR and PAC I through PAC IV (Ad bp 1-321), deleting PAC V and other sequences in the Ad region bp322-360.

These modified helper viruses are characterized by reduced efficiency of helper virus encapsidation. These helper viruses with the specific modifications of the sequences related to packaging efficiency, provide a packaging efficiency high enough for generating production lots of the helper virus, yet low enough that they permit the achievement of higher yields of AdΔ transducing viral particles according to this invention.

B. The Selected Adenovirus Genes

Helper viruses useful in this invention, whether or not they contain the "crippling" modifications described above, contain selected adenovirus gene sequences depending upon the cell line which is transfected by the helper virus and shuttle vector. A preferred helper virus contains a variety of adenovirus genes in addition to the modified sequences described above.

As one example, if the cell line employed to produce the recombinant virus is not a packaging cell line, the helper virus may be a wild type Ad virus. Thus, the helper virus supplies the necessary adenovirus early genes E1, E2, E4 and all remaining late, intermediate, structural and non-structural genes of the adenovirus genome. This helper virus may be a crippled helper virus by incorporating modifications in its native 5' packaging/enhancer domain.

A desirable helper virus is replication defective and lacks all or a sufficient portion of the adenoviral early immediate early gene E1a (which spans mu 1.3 to 4.5) and delayed early gene E1b (which spans mu 4.6 to 11.2) so as to eliminate their normal biological functions. Such replication deficient viruses may also have crippling modifications in the packaging/enhancer domain. Because of the difficulty surrounding the absolute removal of adenovirus from AdΔ preparations that have been enriched by CsCl buoyant density centrifugation, the use of a replication defective adenovirus helper prevents the introduction of infectious adenovirus for *in vivo* animal studies. This helper virus is employed with a packaging cell line which supplies the deficient E1 proteins, such as the 293 cell line.

Additionally, all or a portion of the adenovirus delayed early gene E3 (which spans mu 76.6 to 86.2) may be eliminated from the adenovirus sequence which forms a part of the helper viruses useful in this invention, without adversely affecting the function of the helper virus because this gene product is not necessary for the formation of a functioning virus.

In the presence of other packaging cell lines which are capable of supplying adenoviral proteins in addition to the E1, the helper virus may accordingly be deleted of the genes encoding these adenoviral proteins. Such additionally deleted helper viruses also desirably contain crippling modifications as described above.

C. A Reporter Minigene

It is also desirable for the helper virus to contain a reporter minigene, in which the reporter gene is desirably different from the reporter transgene contained in the shuttle vector. A number of such reporter genes are known, as referred to above. The presence of a reporter gene on the helper virus which is different from the reporter gene on the pAdΔ, allows both the recombinant AdΔ virus and the helper virus to be independently monitored. For example, the expression of recombinant alkaline phosphatase enables residual quantities of contaminating adenovirus to be monitored independent of recombinant LacZ expressed by an pAdΔ shuttle vector or an AdΔ virus.

D. Helper Virus Polycation Conjugates

Still another method for reducing the contamination of helper virus involves the formation of poly-cation helper virus conjugates, which may be associated with a plasmid containing other adenoviral genes, which are not present in the helper virus. The helper viruses described above may be further modified by resort to adenovirus-polylysine conjugate technology.

See, e.g., Wu et al, J. Biol. Chem., 264:16985-16987 (1989); and K. J. Fisher and J. M. Wilson, Biochem. J., 299: 49 (April 1, 1994), incorporated herein by reference.

Using this technology, a helper virus containing preferably the late adenoviral genes is modified by the addition of a poly-cation sequence distributed around the capsid of the helper virus. Preferably, the poly-cation is poly-lysine, which attaches around the negatively-charged vector to form an external positive charge. A plasmid is then designed to express those adenoviral genes not present in the helper virus, e.g., the E1, E2 and/or E4 genes. The plasmid associates to the helper virus-conjugate through the charges on the poly-lysine sequence. This modification is also desirably made to a crippled helper virus of this invention. This conjugate (also termed a trans-infection particle) permits additional adenovirus genes to be removed from the helper virus and be present on a plasmid which does not become incorporated into the virus during production of the recombinant viral vector. Thus, the impact of contamination is considerably lessened.

III. Assembly of Shuttle Vector, Helper Virus and Production of Recombinant Virus

The material from which the sequences used in the pAdΔ shuttle vector and the helper viruses are derived, as well as the various vector components and sequences employed in the construction of the shuttle vectors, helper viruses, and AdΔ viruses of this invention, are obtained from commercial or academic sources based on previously published and described materials. These materials may also be obtained from an individual patient or generated and selected using standard recombinant molecular cloning techniques known and practiced by those

skilled in the art. Any modification of existing nucleic acid sequences forming the vectors and viruses, including sequence deletions, insertions, and other mutations are also generated using standard techniques.

Assembly of the selected DNA sequences of the adenovirus, and the reporter genes or therapeutic genes and other vector elements into the pAdΔ shuttle vector using conventional techniques is described in Example 1 below. Such techniques include conventional cloning techniques of cDNA such as those described in texts [Sambrook et al, cited above], use of overlapping oligonucleotide sequences of the adenovirus genomes, polymerase chain reaction, and any suitable method which provides the desired nucleotide sequence. Standard transfection and co-transfection techniques are employed, e.g., $CaPO_4$ transfection techniques using the HEK 293 cell line. Other conventional methods employed in this invention include homologous recombination of the viral genomes, plaquing of viruses in agar overlay, methods of measuring signal generation, and the like. Assembly of any desired AdΔ vector or helper virus of this invention is within the skill of the art, based on the teachings of this invention.

A. *Shuttle Vector*

As described in detail in Example 1 below and with resort to Fig. 2A and the DNA sequence of the plasmid reported in Fig. 3, a unique pAdΔ shuttle vector of this invention, pAdΔ.CMVLacZ, is generated. pAdΔ.CMVLacZ contains Ad5 sequences encoding the 5' terminal followed by a CMV promoter/enhancer, a splice donor/splice acceptor sequence, a bacterial beta-galactosidase gene (LacZ), a SV-40 poly A sequence (pA), a 3' ITR from Ad5 and remaining plasmid sequence from plasmid pSP72 (Promega) backbone.

To generate the AdΔ genome which is incorporated in the vector, the plasmid pAdΔ.CMVLacZ must be must be digested with EcoRI to release the AdΔ.CMVLacZ genome, freeing the adenovirus ITRs and making them available targets for replication. Thus production of the vector is "restriction-dependent", i.e., requires restriction endonuclease rescue of the replication template. See, Fig. 2B.

A second type of pAdΔ plasmid was designed which places the 3' Ad terminal sequence in a head-to-tail arrangement relative to the 5' terminal sequence. As described in Example 1 and Figs. 4A, and with resort to the DNA sequence of the plasmid reported in Fig. 5, a second unique AdΔ vector sequence of this invention, AdΔc.CMVLacZ, is generated from the shuttle plasmid pAdΔc.CMVLacZ, which contains an Ad5 5' ITR sequence and 3' ITR sequence positioned head-to-tail, followed by a CMV enhancer/ promoter, SD/SA sequence, LacZ gene and pA sequence in a plasmid pSP72 (Promega) backbone. As described in Example 1B, this "restriction-independent" plasmid permits the AdΔ genome to be replicated and rescued from the plasmid backbone without including an endonuclease treatment (see, Fig. 4B).

B. Helper Virus

As described in detail in Example 2, an exemplary conventional E1 deleted adenovirus helper virus is virus Ad.CBhpAP, which contains a 5' adenovirus sequence from mu 0-1, a reporter minigene containing human placenta alkaline phosphatase (hpAP) under the transcriptional control of the chicken ß-actin promoter, followed by a poly-A sequence from SV40, followed by adenovirus sequences from 9.2 to 78.4 and 86 to 100. This helper contained deletions from mu 1.0 to 9.2 and 78.4 to 86, which eliminate substantially the E1 region and the E3 region of the virus. This virus may be

desirably crippled according to this invention by modifications to its packaging enhancer domain.

Exemplary crippled helper viruses of this invention are described using the techniques described in Example 7 and contain the modified 5' PAC sequences, i.e., adenovirus genome bp 1-269; m.u. 0-0.75 or adenovirus genome bp 1-321; m.u. 0-0.89. Briefly, the 5' sequences are modified by PCR and cloned by conventional techniques into a conventional adenovirus based plasmid. A hpAP minigene is incorporated into the plasmid, which is then altered by homologous recombination with an E3 deleted adenovirus dl7001 to result in the modified vectors so that the reporter minigene is followed on its 3' end with the adenovirus sequences mu 9.6 to 78.3 and 87 to 100.

Generation of a poly-L-lysine conjugate helper virus was demonstrated essentially as described in detail in Example 5 below and Fig. 10 by coupling poly-L-lysine to the Ad.CBhpAP virion capsid. Alternatively, the same procedure may be employed with the PAC sequence modified helper viruses of this invention.

C. Recombinant AdΔ Virus

As stated above, a pAdΔ shuttle vector in the presence of helper virus and/or a packaging cell line permits the adenovirus-transgene sequences in the shuttle vector to be replicated and packaged into virion capsids, resulting in the recombinant AdΔ virus. The current method for producing such AdΔ virus is transfection-based and described in detail in Example 3. Briefly, helper virus is used to infect cells, such as the packaging cell line human HEK 293, which are then subsequently transfected with an pAdΔ shuttle vector containing a selected transgene by conventional methods. About 30 or more hours post-transfection, the cells are harvested, and an extract prepared. The AdΔ viral genome is

packaged into virions that sediment at a lower density than the helper virus in cesium gradients. Thus, the recombinant AdΔ virus containing a selected transgene is separated from the bulk of the helper virus by purification via buoyant density ultracentrifugation in a CsCl gradient.

The yield of AdΔ transducing virus is largely dependent on the number of cells that are transfected with the pAdΔ shuttle plasmid, making it desirable to use a transfection protocol with high efficiency. One such method involves use of a poly-L-lysinylated helper adenovirus as described above. A pAdΔ shuttle plasmid containing the desired transgene under the control of a suitable promoter, as described above, is then complexed directly to the positively charged helper virus capsid, resulting in the formation of a single transfection particle containing the pAdΔ shuttle vector and the helper functions of the helper virus.

The underlying principle is that the helper adenovirus coated with plasmid pAdΔ DNA will co-transport the attached nucleic acid across the cell membrane and into the cytoplasm according to its normal mechanism of cell entry. Therefore, the poly-L-lysine modified helper adenovirus assumes multiple roles in the context of an AdΔ-based complex. First, it is the structural foundation upon which plasmid DNA can bind increasing the effective concentration. Second, receptor mediated endocytosis of the virus provides the vehicle for cell uptake of the plasmid DNA. Third, the endosomalytic activity associated with adenoviral infection facilitates the release of internalized plasmid into the cytoplasm. And the adenovirus contributes trans helper functions on which the recombinant AdΔ virus is dependent for replication and packaging of transducing viral particles. The Ad-based transfection procedure using an pAdΔ shuttle

vector and a polycation-helper conjugate is detailed in Example 6. Additionally, as described previously, the helper virus-plasmid conjugate may be another form of helper virus delivery of the omitted adenovirus genes not present in the pAdΔ vector. Such a structure enables the rest of the required adenovirus genes to be divided between the plasmid and the helper virus, thus reducing the self-replication efficiency of the helper virus.

A presently preferred method of producing the recombinant AdΔ virus of this invention involves performing the above-described transfection with the crippled helper virus or crippled helper virus conjugate, as described above. A "crippled" helper virus of this invention is unable to package itself efficiently, and therefor permits ready separation of the helper virus from the newly packaged AdΔ vector of this invention by use of buoyant density ultracentrifugation in a CsCl gradient, as described in the examples below.

IV. Function of the Recombinant AdΔ Virus

Once the AdΔ virus of this invention is produced by cooperation of the shuttle vector and helper virus, the AdΔ virus can be targeted to, and taken up by, a selected target cell. The selection of the target cell also depends upon the use of the recombinant virus, i.e., whether or not the transgene is to be replicated *in vitro* or *ex vivo* for production in a desired cell type for redelivery into a patient, or *in vivo* for delivery to a particular cell type or tissue. Target cells may be any mammalian cell (preferably a human cell). For example, in *in vivo* use, the recombinant virus can target to any cell type normally infected by adenovirus, depending upon the route of administration, i.e., it can target, without limitation, neurons, hepatocytes, epithelial cells and

the like. The helper adenovirus sequences supply the sequences necessary to permit uptake of the virus by the AdΔ.

Once the recombinant virus is taken up by a cell, the adenovirus flanked transgene is rescued from the parental adenovirus backbone by the machinery of the infected cell, as with other recombinant adenoviruses. Once uncoupled (rescued) from the genome of the AdΔ virus, the recombinant minigene seeks an integration site in the host chromatin and becomes integrated therein, either transiently or stably, providing expression of the accompanying transgene in the host cell.

V. Use of the AdΔ Viruses in Gene Therapy

The novel recombinant viruses and viral conjugates of this invention provide efficient gene transfer vehicles for somatic gene therapy. These viruses are prepared to contain a therapeutic gene in place of the *LacZ* reporter transgene illustrated in the exemplary viruses and vectors. By use of the AdΔ viruses containing therapeutic transgenes, these transgenes can be delivered to a patient *in vivo* or *ex vivo* to provide for integration of the desired gene into a target cell. Thus, these viruses can be employed to correct genetic deficiencies or defects. An example of the generation of an AdΔ gene transfer vehicle for the treatment of cystic fibrosis is described in Example 4 below. One of skill in the art can generate any number of other gene transfer vehicles by including a selected transgene for the treatment of other disorders.

The recombinant viruses of the present invention may be administered to a patient, preferably suspended in a biologically compatible solution or pharmaceutically acceptable delivery vehicle. A suitable vehicle includes sterile saline. Other aqueous and non-aqueous isotonic

sterile injection solutions and aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carriers and well known to those of skill in the art may be employed for this purpose.

The recombinant viruses of this invention may be administered in sufficient amounts to transfect the desired cells and provide sufficient levels of integration and expression of the selected transgene to provide a therapeutic benefit without undue adverse effects or with medically acceptable physiological effects which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable parenteral routes of administration include direct delivery to the target organ, tissue or site, intranasal, intravenous, intramuscular, subcutaneous, intradermal and oral administration. Routes of administration may be combined, if desired.

Dosages of the recombinant virus will depend primarily on factors such as the condition being treated, the selected gene, the age, weight and health of the patient, and may thus vary among patients. A therapeutically effective human dosage of the viruses of the present invention is believed to be in the range of from about 20 to about 50 ml of saline solution containing concentrations of from about 1×10^7 to 1×10^{10} pfu/ml virus of the present invention. A preferred human dosage is about 20 ml saline solution at the above concentrations. The dosage will be adjusted to balance the therapeutic benefit against any side effects. The levels of expression of the selected gene can be monitored to determine the selection, adjustment or frequency of dosage administration.

The following examples illustrate the construction of the pAdΔ shuttle vectors, helper viruses and recombinant AdΔ viruses of the present invention and the use thereof in gene therapy. These examples are illustrative only, and do not limit the scope of the present invention.

Example 1 - Production of pAdΔ.CMVLacZ and pAdΔc.CMVLacZ Shuttle Vectors

A. pAdΔ.CMVLacZ

A human adenovirus Ad5 sequence was modified to contain a deletion in the E1a region [map units 1 to 9.2], which immediately follows the Ad 5' region (bp 1-360) (illustrated in Figs. 1A). Thus, the plasmid contains the 5' ITR sequence (bp 1-103), the native packaging/enhancer sequences and the TATA box for the E1a region (bp 104-360). A minigene containing the CMV immediate early enhancer/promoter, an SD/SA sequence, a cytoplasmic lacZ gene, and SV40 poly A (pA), was introduced at the site of the E1a deletion. This construct was further modified so that the minigene is followed by the 3' ITR sequences (bp 35,353-end). The DNA sequences for these components are provided in Fig. 3 and SEQ ID NO: 1 (see, also the brief description of this figure).

This construct was then cloned by conventional techniques into a pSP72 vector (Promega) backbone to make the circular shuttle vector pAdΔCMVLacZ. See the schematic of Fig. 2A. This construct was engineered with EcoRI sites flanking the 5' and 3' Ad5 ITR sequences. pAdΔ.CMVLacZ was then subjected to enzymatic digestion with EcoRI, releasing a linear fragment of the vector spanning the terminal end of the Ad 5'ITR sequence through the terminal end of the 3'ITR sequence from the plasmid backbone. See Fig. 2B.

B. pAdΔc.CMVLacZ

The shuttle vector pAdΔc.CMVLacZ (Figs. 4A and 5) was constructed using a pSP72 (Promega) backbone so that the Ad5 5' ITR and 3' ITR were positioned head-to-tail. The organization of the Ad5 ITRs was based on reports that suggest circular Ad genomes that have the terminal ends fused together head-to-tail are infectious to levels comparable to linear Ad genomes. A minigene encoding the CMV enhancer, an SD/SA sequence, the LacZ gene, and the poly A sequence was inserted immediately following the 5' ITR. The DNA sequence of the resulting plasmid and the sequences for the individual components are reported in Fig. 5 and SEQ ID NO: 2 (see also, brief description of Fig. 5). This plasmid does not require enzymatic digestion prior to its use to produce the viral particle (see Example 3). This vector was designed to enable restriction-independent production of LacZ AdΔ vectors.

Example 2 - Construction of a Helper Virus

The Ad.CBhpAP helper virus [K. Kozarsky et al, Som. Cell Mol. Genet., 19(5):449-458 (1993)] is a replication deficient adenovirus containing an alkaline phosphatase minigene. Its construction involved conventional cloning and homologous recombination techniques. The adenovirus DNA substrate was extracted from CsCl purified d17001 virions, an Ad5 (serotype subgroup C) variant that carries a 3 kb deletion between mu 78.4 through 86 in the nonessential E3 region (provided by Dr. William Wold, Washington University, St. Louis, Missouri). Viral DNA was prepared for co-transfection by digestion with ClaI (adenovirus genomic bp position 917) which removes the left arm of the genome encompassing adenovirus map units 0-2.5. See lower diagram of Fig. 1B.

A parental cloning vector, pAd.BglII was designed. It contains two segments of wild-type Ad5 genome (i.e., map units 0-1 and 9-16.1) separated by a unique BglII cloning site for insertion of heterologous sequences. The missing Ad5 sequences between the two domains (adenovirus genome bp 361-3327) results in the deletion of E1a and the majority of E1b following recombination with viral DNA.

A recombinant hpAP minigene was designed and inserted into the BglII site of pAd.BglII to generate the complementing plasmid, pAdCBhpAP. The linear arrangement of this minigene includes:

(a) the chicken cytoplasmic ß-actin promoter [nucleotides +1 to +275 as described in T. A. Kost et al, Nucl. Acids Res., 11(23):8287 (1983); nucleotides 9241-8684 of Fig. 7];

(b) an SV40 intron (e.g., nucleotides 1579-1711 of SEQ ID NO: 2),

(c) the sequence for human placental alkaline phosphatase (available from Genbank) and

(d) an SV40 polyadenylation signal (a 237 Bam HI-BclI restriction fragment containing the cleavage/poly-A signals from both the early and late transcription units; e.g., nucleotides 837-639 of SEQ ID NO: 1).

The resulting complementing plasmid, pAdCBhpAP contained a single copy of recombinant hpAP minigene flanked by adenovirus coordinates 0-1 on one side and 9.2-16.1 on the other.

Plasmid DNA was linearized using a unique NheI site immediately 5' to adenovirus map unit zero (0) and the above-identified adenovirus substrate and the complementing plasmid DNAs were transfected to 293 cells [ATCC CRL1573] using a standard calcium phosphate transfection procedure [see, e.g., Sambrook et al, cited above]. The end result of homologous recombination

involving sequences that map to adenovirus map units 9-16.1 is hybrid Ad.CBhpAP helper virus which contains adenovirus map units 0-1 and, in place of the E1a and E1b coding regions from the d17001 adenovirus substrate, is the hpAP minigene from the plasmid, followed by Ad sequences 9 to 100, with a deletion in the E3 (78.4-86 mu) regions.

Example 3 - Production of Recombinant AdΔ Virus

The recombinant AdΔ virus of this invention are generated by co-transfection of a shuttle vector with the helper virus in a selected packaging or non-packaging cell line.

As described in detail below, the linear fragment provided in Example 1A, or the circular AdΔ genome carrying the LacZ of Example 1B, is packaged into the Ad.CBhpAP helper virus (Example 2) using conventional techniques, which provides an empty capsid head, as illustrated in Fig. 2C. Those virus particles which have successfully taken up the pAd shuttle genome into the capsid head can be distinguished from those containing the hpAP gene by virtue of the differential expression of LacZ and hpAP.

In more detail, 293 cells (4 x 10^7 pfu 293 cells/150 mm dish) were seeded and infected with helper virus Ad.CBhpAP (produced as described in Example 2) at an MOI of 5 in 20 ml DMEM/2% fetal bovine serum (FBS). This helper specific marker is critical for monitoring the level of helper virus contamination in AdΔ preparations before and after purification. The helper virus provides in trans the necessary helper functions for synthesis and packaging of the AdΔCMV*LacZ* genome.

Two hours post infection, using either the restriction-dependent shuttle vector or the restriction-independent shuttle vector, plasmid pAdΔ.CMVLacZ

(digested with EcoRI) or pAdAc.CMVLacZ DNA, each carrying a LacZ minigene, was added to the cells by a calcium phosphate precipitate (2.5 ml calcium phosphate transfection cocktail containing 50 μg plasmid DNA).

Thirty to forty hours post-transfection, cells were harvested, suspended in 10 mM Tris-Cl (pH 8.0) (0.5 ml/150 mm plate) and frozen at -80°C. Frozen cell suspensions were subjected to three rounds of freeze (ethanol-dry ice)-thaw (37°C) cycles to release virion capsids. Cell debris was removed by centrifugation (5,000xg for 10 minutes) and the clarified supernatant applied to a CsCl gradients to separate recombinant virus from helper virus as follows.

Supernatants (10 ml) applied to the discontinuous CsCl gradient (composed of equal volumes of CsCl at 1.2 g/ml, 1.36 g/ml, and 1.45 g/ml 10 mM Tris-Cl (pH 8.0)) were centrifuged for 8 hours at 72,128Xg, resulting in separation of infectious helper virus from incompletely formed virions. Fractions were collected from the interfacing zone between the helper and top components and analyzed by Southern blot hybridization or for the presence of LacZ transducing particles. For functional analysis, aliquots (2.0 ml from each sample) from the same fractions were added to monolayers of 293 cells (in 35 mm wells) and expression of recombinant ß-galactosidase determined 24 hours later. More specifically, monolayers were harvested, suspended in 0.3 ml 10 mM Tris-Cl (pH 8.0) buffer and an extract prepared by three rounds of freeze-thaw cycles. Cell debris was removed by centrifugation and the supernatant tested for ß-galactosidase (LacZ) activity according to the procedure described in J. Price et al, Proc. Natl. Acad. Sci., USA, 84:156-160 (1987). The specific activity (milliunits ß-galactosidase/mg protein or reporter

enzymes was measured from indicator cells. For the recombinant virus, specific activity was 116.

Fractions with ß-galactosidase activity from the discontinuous gradient were sedimented through an equilibrium cesium gradient to further enrich the preparation for Adᐃ virus. A linear gradient was generated in the area of the recombinant virus spanning densities 1.29 to 1.34gm/ml. A sharp peak of the recombinant virus, detected as the appearance of the ß-gal activity in infected 293 cells, eluted between 1.31 and 1.33 gm/dl. This peak of recombinant virus was located between two major A_{260} nm absorbing peaks and in an area of the gradient with the helper virus was precipitously dropping off. The equilibrium sedimentation gradient accomplished another 102 to 103 fold purification of recombinant virus from helper virus. The yield of recombinant Adᐃ.CMVLacZ virus recovered from a 50 plate prep after 2 sedimentations ranged from 107 to 108 transducing particles.

Analysis of lysates of cells transfected with the recombinant vector and infected with helper revealed virions capable of transducing the recombinant minigene contained within the vector. Subjecting aliquots of the fractions to Southern analysis using probes specific to the recombinant virus or helper virus revealed packaging of multiple molecular forms of vector derived sequence. The predominant form of the deleted viral genome was the size (~5.5 kb) of the corresponding double stranded DNA monomer (Adᐃ.CMVLacZ) with less abundant but discrete higher molecular weight species (~10 kb and ~15 kb) also present. Full-length helper virus is 35kb. Importantly, the peak of vector transduction activity corresponds with the highest molecular weight form of the deleted virus. These results confirm the hypothesis that ITRs and contiguous packaging sequence are the only elements

necessary for incorporation into virions. An apparently ordered or preferred rearrangement of the recombinant Ad monomer genome leads to a more biologically active molecule. The fact that larger molecular species of the deleted genome are 2x and 3x fold larger than the monomer deleted virus genome suggests that the rearrangements may involve sequential duplication of the original genome.

These same procedures may be adapted for production of a recombinant AdΔ virus using a crippled helper virus or helper virus conjugate as described previously.

Example 4 - Recombinant AdΔ Virus Containing a Therapeutic Minigene

To test the versatility of the recombinant AdΔ virus system, the reporter LacZ minigene obtained from pAdΔCMVLacZ was cassette replaced with a therapeutic minigene encoding CFTR.

The minigene contained human CFTR cDNA [Riordan et al, Science, 245:1066-1073 (1989); nucleotides 8622-4065 of SEQ ID NO: 3] under the transcriptional control of a chimeric CMV enhancer/chicken ß-actin promotor element (nucleotides +1 to +275 as described in T. A. Kost et al, Nucl. Acids Res., 11(23):8287 (1983); nucleotides 9241-8684 of SEQ ID NO: 3, Fig. 7); and followed by an SV-40 poly-A sequence (nucleotides 3887-3684 of SEQ ID NO: 3, Fig. 7).

The CFTR minigene was inserted into the E1 deletion site of an Ad5 virus (called pAd.E1Δ) which contains a deletion in E1a from mu 1-9.2 and a deletion in E3 from mu 78.4-86.

The resulting shuttle vector called pAdΔ.CBCFTR (see Figs. 6 and the DNA sequence of Fig. 7 [SEQ ID NO: 3]) used the same Ad ITRs of pAdΔCMVLacZ, but the Ad5 sequences terminated with NheI sites instead of EcoRI.

Therefore release of the minigene from the plasmid was accomplished by digestion with NheI.

The vector production system described in Example 3 was employed, using the helper virus Ad.CBhpAP (Example 2). Monolayers of 293 cells grown to 80-90% confluency in 150 mm culture dishes were infected with the helper virus at an MOI of 5. Infections were done in DMEM supplemented with 2% FBS at 20 ml media/150 mm plate. Two hours post-infection, 50 μg plasmid DNA in 2.5 ml transfection cocktail was added to each plate and evenly distributed.

Delivery of the pAdΔ.CBCFTR plasmid to 293 cells was mediated by formation of a calcium phosphate precipitate and AdΔ.CBCFTR virus resolved from Ad.CBhpAP helper virus by CsCl buoyant density ultracentrifugation as follows:

Cells were left in this condition for 10-14 h, afterwhich the infection/transfection media was replaced with 20 ml fresh DMEM/2% FBS. Approximately 30 h post-transfection, cells were harvested, suspended in 10 mM Tris-Cl (pH 8.0) buffer (0.5 ml/150 mm plate), and stored at -80°C.

Frozen cell suspensions were lysed by three sequential rounds of freeze (ethanol-dry ice)-thaw (37°C). Cell debris was removed by centrifugation (5,000 x g for 10 min) and 10 ml clarified extract layered onto a CsCl step gradient composed of three 9.0 ml tiers with densities 1.45 g/ml, 1.36 g/ml, and 1.20 g/ml CsCl in 10 mM Tris-Cl (pH 8.0) buffer. Centrifugation was performed at 20,000 rpm in a Beckman SW-28 rotor for 8 h at 4°C. Fractions (1.0 ml) were collected from the bottom of the centrifuge tube and analyzed for rΔAd transducing vectors. Peak fractions were combined and banded to equilibrium. Fractions containing transducing virions were dialyzed against 20 mM HEPES (pH 7.8)/150 mM NaCl

(HBS) and stored frozen at -80°C in the presence of 10% glycerol or as a liquid stock at -20°C (HBS+40% glycerol).

Fractions collected after ultracentrifugation were analyzed for transgene expression and vector DNA. For *lacZ* ΔrAd vectors, 2 μl aliquots were added to 293 cell monolayers seeded in 35 mm culture wells. Twenty-four hours later cells were harvested, suspended in 0.3 ml 10 mM Tris-Cl (pH 8.0) buffer, and lysed by three rounds of freeze-thaw. Cell debris was removed by centrifugation (15,000 x g for 10 min) and assayed for total protein [Bradford, (1976)] and β-galactosidase activity [Sambrook et al, (1989)] using ONPG (o-Nitrophenyl β-D-galactopyranoside) as substrate.

Expression of CFTR protein from the AdΔ.CBCFTR vector was determined by immunofluorescence localization. Aliquots of AdΔ.CBCFTR, enriched by two-rounds of ultracentrifugation and exchanged to HBS storage buffer, were added to primary cultures of airway epithelial cells obtained from the lungs of CF transplant recipients. Twenty-four hours after the addition of vector, cells were harvested and affixed to glass slides using centrifugal force (Cytospin 3, Shandon Scientific Limited). Cells were fixed with freshly prepared 3% paraformaldehyde in PBS (1.4 mM KH_2PO_4, 4.3 mM Na_2HPO_4, 2.7 mM KCl, and 137 mM NaCl) for 15 min at room temperature (RT), washed twice in PBS, and permeabilized with 0.05% NP-40 for 10 min at RT. The immunofluorescence procedure began with a blocking step in 10% goat serum (PBS/GS) for 1 h at RT, followed by binding of the primary monoclonal mouse anti-human CFTR (R-domain specific) antibody (Genzyme) diluted 1:500 in PBS/GS for 2 h at RT. Cells were washed extensively in PBS/GS and incubated for 1 h at RT with a donkey anti-mouse IgG (H+L) FITC conjugated

antibody (Jackson ImmunoResearch Laboratories) diluted 1:100 in PBS/GS.

For Southern analysis of vector DNA, 5 µl aliquots were taken directly from CsCl fractions and incubated with 20 µl capsid digestion buffer (50 mM Tris-Cl, pH 8.0; 1.0 mM EDTA, pH 8.0; 0.5% SDS, and 1.0 mg/ml Proteinase K) at 50°C for 1 h. The reactions were allowed to cool to RT, loading dye was added, and electrophoresed through a 1.2% agarose gel. Resolved DNAs were electroblotted onto a nylon membrane (Hybond-N) and hybridized with a 32-P labeled restriction fragment. Blots were analyzed by autoradiography or scanned on a Phosphorimager 445 SI (Molecular Dynamics).

The results that were obtained from Southern blot analysis of gradient fractions revealed a distinct viral band that migrated faster than the helper Ad.CBhpAP DNA. The highest viral titers mapped to fractions 3 and 4. Quantitation of the bands in fraction 4 indicated the titer of Ad.CBhpAP was approximately 1.5x greater than AdΔCBCFTR. However, if the size difference between the two viruses is factored in (Ad.CBhpAP=35 kb; AdΔCBCFTR=6.2 kb), the viral titer (where 1 particle=1 DNA molecule) of AdΔCB.CFTR is at least 4-fold greater than the viral titer of Ad.CBhpAP.

While Southern blot analysis of gradient fractions was useful for showing the production of AdΔ viral particles, it also demonstrated the utility of ultracentrifugation for purifying AdΔ viruses. Considering the latter of these, both LacZ and CFTR transducing viruses banded in CsCl to an intermediate density between infectious adenovirus helper virions (1.34 g/ml) and incompletely formed capsids (1.31 g/ml). The lighter density relative to helper virus likely results from the smaller genome carried by the AdΔ viruses. This further suggests changes in virus size

influences the density and purification of AdΔ virus. Regardless, the ability to separate AdΔ virus from the helper virus is an important observation and suggests further purification may be achieved by successive rounds of banding through CsCl.

This recombinant virus is useful in gene therapy alone, or preferably, in the form of a conjugate prepared as described herein.

Example 5 - Correction of Genetic Defect in CF airway Epithelial Cells with AdΔCB.CFTR

Treatment of cystic fibrosis, utilizing the recombinant virus provided above, is particularly suited for *in vivo*, lung-directed, gene therapy. Airway epithelial cells are the most desirable targets for gene transfer because the pulmonary complications of CF are usually its most morbid and life-limiting.

The recombinant AdΔCB.CFTR virus was fractionated on sequential CsCl gradients and fractions containing CFTR sequences, migrating between the adenovirus and top components fractions described above were used to infect primary cultures of human airway epithelial cells derived from the lungs of a CF patient. The cultures were subsequently analyzed for expression of CFTR protein by immunocytochemistry. Immunofluorescent detection with mouse anti-human CFTR (R domain specific) antibody was performed 24 hours after the addition of the recombinant virus. Analysis of mock infected CF cells failed to reveal significant binding to the R domain specific CFTR antibody. Primary airway epithelium cultures exposed to the recombinant virus demonstrated high levels of CFTR protein in 10-20% of the cells.

Thus, the recombinant virus of the invention, containing the CFTR gene, may be delivered directly into the airway, e.g. by a formulating the virus above, into a

preparation which can be inhaled. For example, the recombinant virus or conjugate of the invention containing the CFTR gene, is suspended in 0.25 molar sodium chloride. The virus or conjugate is taken up by respiratory airway cells and the gene is expressed.

Alternatively, the virus or conjugates of the invention may be delivered by other suitable means, including site-directed injection of the virus bearing the CFTR gene. In the case of CFTR gene delivery, preferred solutions for bronchial instillation are sterile saline solutions containing in the range of from about 1×10^7 to 1×10^{10} pfu/ml, more particularly, in the range of from about 1×10^8 to 1×10^9 pfu/ml of the virus of the present invention.

Other suitable methods for the treatment of cystic fibrosis by use of gene therapy recombinant viruses of this invention may be obtained from the art discussions of other types of gene therapy vectors for CF. See, for example, U. S. Patent No. 5,240,846, incorporated by reference herein.

Example 6 - Synthesis of Polycation Helper Virus Conjugate

Another version of the helper virus of this invention is a polylysine conjugate which enables the pAdΔ shuttle plasmid to complex directly with the helper virus capsid. This conjugate permits efficient delivery of shuttle plasmid pAdΔ shuttle vector in tandem with the helper virus, thereby removing the need for a separate transfection step. See, Fig. 10 for a diagrammatic outline of this construction. Alternatively, such a conjugate with a plasmid supplying some Ad genes and the helper supplying the remaining necessary genes for production of the AdΔ viral vector provides a novel way

to reduce contamination of the helper virus, as discussed above.

Purified stocks of a large-scale expansion of Ad.CBhpAP were modified by coupling poly-L-lysine to the virion capsid essentially as described by K. J. Fisher and J. M. Wilson, Biochem. J., 299:49-58 (1994), resulting in an Ad.CBhpAP-$(Lys)_n$ conjugate. The procedure involves three steps.

First, CsCl band purified helper virus Ad.CBhpAP was reacted with the heterobifunctional crosslinker sulfo-SMCC [sulfo-(N-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate] (Pierce). The conjugation reaction, which contained 0.5 mg (375 nmol) of sulpho-SMCC and 6 x 10^{12} A_{260} helper virus particles in 3.0 ml of HBS, was incubated at 30°C for 45 minutes with constant gentle shaking. This step involved formation of a peptide bond between the active N-hydroxysuccinimide (NHS) ester of sulpho-SMCC and a free amine (e.g. lysine) contributed by an adenovirus protein sequence (capsid protein) in the vector, yielding a maleimide-activated viral particle. The activated adenovirus is shown in Fig. 10 having the capsid protein fiber labeled with the nucleophilic maleimide moiety. In practice, other capsid polypeptides including hexon and penton base are also targeted.

Unincorporated, unreacted cross-linker was removed by gel filtration on a 1 cm x 15 cm Bio-Gel P-6DG (Bio-Rad Laboratories) column equilibrated with 50 mM Tris/HCl buffer, pH 7.0, and 150 mM NaCl. Peak A_{260} fractions containing maleimide-activated helper virus were combined and placed on ice.

Second, poly-L-lysine having a molecular mass of 58 kDa at 10 mg/ml in 50 mM triethanolamine buffer (pH 8.0), 150 mM NaCl and 1 mM EDTA was thiolated with 2-imminothiolane/HCl (Traut's Reagent; Pierce) to a molar

ratio of 2 moles -SH/mole polylysine under N_2; the cyclic thioimidate reacts with the poly(L-lysine) primary amines resulting in a thiolated polycation. After a 45 minute incubation at room temperature the reaction was applied to a 1 cm x 15 cm Bio-Gel P6DG column equilibrated with 50 mM Tris/HCl buffer (pH 7.0), 150 mM NaCl and 2 mM EDTA to remove unincorporated Traut's Reagent.

Quantification of free thiol groups was accomplished with Ellman's reagent [5,5'-dithio-bis-(2-nitrobenzoic acid)], revealing approximately 3-4 mol of -SH/mol of poly(L-lysine). The coupling reaction was initiated by adding 1 x 10^{12} A_{260} particles of maleimide-activated helper virus/mg of thiolated poly(L-lysine) and incubating the mixture on ice at 4°C for 15 hours under argon. 2-mercaptoethylamine was added at the completion of the reaction and incubation carried out at room temperature for 20 minutes to block unreacted maleimide sites.

Virus-polylysine conjugates, Ad.CPAP-p$(Lys)_n$, were purified away from unconjugated poly(L-lysine) by ultracentrifugation through a CsCl step gradient with an initial composition of equal volumes of 1.45 g/ml (bottom step) and 1.2 g/ml (top step) CsCl in 10 mM Tris/HCl buffer (pH 8.0). Centrifugation was at 90,000 g for 2 hours at 5°C. The final product was dialyzed against 20 mM Hepes buffer (pH 7.8) containing 150 mM NaCl (HBS).

Example 7 - Formation of AdΔ/helper-pLys Viral Particle

The formation of Ad.CBhpAP-pLys/pAdΔ.CMVLacZ particle is initiated by adding 20 μg plasmid pAdΔ.CMVLacZ DNAs to 1.2 x 10^{12} A_{260} particles Ad.CBhpAP-pLys in a final volume of 0.2 ml DMEM and allowing the complex to develop at room temperature for between 10-15 minutes. This ratio typically represents the plasmid DNA binding capacity of a standard lot of adenovirus-pLys

conjugate and gives the highest levels of plasmid transgene expression.

The resulting trans-infection particle is transfected onto 293 cells (4 x 10^7 cells seeded on a 150 mm dish). Thirty hours after transfection, the particles are recovered and subjected to a freeze/thaw technique to obtain an extract. The extract is purified on a CsCl step gradient with gradients at 1.20 g/ml, 1.36 g/ml and 1.45 g/ml. After centrifugation at 90,000 x g for 8 hours, the AdΔ vectors were obtained from a fraction under the top components as identified by the presence of LacZ, and the helper virus was obtained from a smaller, denser fraction, as identified by the presence of hpAP.

Example 8 - Construction of Modified Helper Viruses with Crippled Packaging (PAC) Sequences

This example refers to Figs. 9A through 9C, 10A and 10B in the design of modified helper viruses of this invention.

Ad5 5' terminal sequences that contained PAC domains I and II (Fig. 8A) or PAC domains I, II, III, and IV (Fig. 8B) were generated by PCR from the wild type Ad5 5' genome depicted in Fig. 1B using PCR clones indicated by the arrows in Fig. 1B. The resulting amplification products (Fig. 8A and 8B) sequences differed from the wild-type Ad5 genome in the number of A-repeats carried by the left (5') end.

As depicted in Fig. 8C, these amplification products were subcloned into the multiple cloning site of pAd.Link.1 (IHGT Vector Core). pAd.Link.1 is a adenovirus based plasmid containing adenovirus m.u. 9.6 through 16.1. The insertion of the modified PAC regions into pAd.Link.1 generated two vectors pAd.PACII (containing PAC domains I and II) and pAd.PACIV (containing PAC domains I, II, III, and IV).

Thereafter, as depicted in Figs. 10A and 10B, for each of these plasmids, a human placenta alkaline phosphatase reporter minigene containing the immediate early CMV enhancer/promoter (CMV), human placenta alkaline phosphatase cDNA (hpAP), and SV40 polyadenylation signal (pA), was subcloned into each PAC vector, generating pAd.PACII.CMVhpAP and pAd.PACIV.CMVhpAP, respectively.

These plasmids were then used as substrates for homologous recombination with dl7001 virus, described above, by co-transfection into 293 cells. Homologous recombination occurred between the adenovirus map units 9-16 of the plasmid and the crippled Ad5 virus. The results of homologous recombination were helper viruses containing Ad5 5' terminal sequences that contained PAC domains I and II or PAC domains I, II, III, and IV, followed by the minigene, and Ad5 3' sequences 9.6-78.3 and 87-100. Thus, these crippled viruses are deleted of the E1 gene and the E3 gene.

The plaque formation characteristics of the PAC helper viruses gave an immediate indication that the PAC modifications diminished the rate and extent of growth. Specifically, PAC helper virus plaques did not develop until day 14-21 post-transfection, and on maturation remained small. From previous experience, a standard first generation Ad.CBhpAP helper virus with a complete left terminal sequence would begin to develop by day 7 and mature by day 10.

Viral plaques were picked and suspended in 0.5 ml of DMEM media. A small aliquot of the virus stock was used to infect a fresh monolayer of 293 cells and histochemically stained for recombinant alkaline phosphatase activity 24 hours post-infection. Six of eight Ad.PACIV.CMVhpAP (encodes A-repeats I-IV) clones that were screened for transgene expression were

positive, while all three Ad.PACII.CMVhpAP clones that were selected scored positive. The clones have been taken through two rounds of plaque purification and are currently being expanded to generate a working stock.

These crippled helper viruses are useful in the production of the AdΔ virus particles according to the procedures described in Example 3. They are characterized by containing sufficient adenovirus genes to permit the packaging of the shuttle vector genome, but their crippled PAC sequences reduce their efficiency for self-encapsidation. Thus less helper viruses are produced in favor of more AdΔ recombinant viruses. Purification of AdΔ virus particles from helper viruses is facilitated in the CsCl gradient, which is based on the weight of the respective viral particles. This facility in purification is a decided advantage of the AdΔ vectors of this invention in contrast to adenovirus vectors having only E1 or smaller deletions. The AdΔ vectors even with minigenes of up to about 15 kb are significantly different in weight than wild type or other adenovirus helpers containing many adenovirus genes.

Example 9 - AdΔ Vector Containing a full-length dystrophin transgene

Duchenne muscular dystrophy (DMD) is a common x-linked genetic disease caused by the absence of dystrophin, a 427K protein encoded by a 14 kilobase transcript. Lack of this important sarcolemmal protein leads to progressive muscle wasting, weakness, and death. One current approach for treating this lethal disease is to transfer a functional copy of the dystrophin gene into the affected muscles. For skeletal muscle, a replication-defective adenovirus represents an efficient delivery system.

According to the present invention, a recombinant plasmid pAdΔ.CMVmdys was created which contains only the Ad5 cis-elements (i.e., ITRs and contiguous packaging sequences) and harbors the full-length murine dystrophin gene driven by the CMV promoter. This plasmid was generated as follows.

pSL1180 [Pharmacia Biotech] was cut with *Not* I, filled in by Klenow, and religated thus ablating the *Not* I site in the plasmid. The resulting plasmid is termed pSL1180NN and carries a bacterial ori and *Amp* resistance gene.

pAdΔ.CMVLacZ of Example 1 was cut with EcoRI, klenowed, and ligated with the ApaI-cut pSL1180NN to form pAdΔ.CMV*LacZ* (ApaI).

The 14 kb mouse dystrophin cDNA [sequences provided in C. C. Lee et al, Nature, 349:334-336 (1991)] was cloned in two large fragments using a lambda ZAP cloning vector (Stratagene) and subsequently cloned into the bluescript vector pSK- giving rise to the plasmid pCCL-DMD. A schematic diagram of this vector is provided in Fig. 11, which illustrates the restriction enzyme sites.

pAdΔ.CMV*LacZ* (ApaI) was cut with *Not*I and the large fragment gel isolated away from the lacZ cDNA. pCCL-DMD was also cut with *Not*I, gel isolated and subseqently ligated to the large NotI fragment of NotI digested pAdΔ.CMVLacZ (ApaI). The sequences of resulting vector, pAdΔ.CMVmdys, are provided in Fig. 12A-12P [SEQ ID NO:10].

This plasmid contains sequences form the left-end of the Ad5 encompassing bp 1-360 (5' ITR), a mouse dystrophin minigene under the control of the CMV promoter, and sequence from the right end of Ad5 spanning

bp 35353 to the end of the genome (3' ITR). The minigene is followed by an SV-40 poly-A sequence similar to that described for the plasmids described above.

The vector production system described herein is employed. Ten 150mm 293 plates are infected at about 90% confluency with a reporter recombinant E1-deleted virus Ad.CBhpAP at an MOI of 5 for 60 minutes at 37°C. These cells are transfected with pAdΔ.CMVmDys by calcium phosphate co-precipitation using 50 μg linearized DNA/dish for about 12-16 hours at 37°C. Media is replaced with DMEM + 10% fetal bovine serum.

Full cytopathic effect is observed and a cell lysate is made by subjecting the cell pellet to freeze-thaw procedures three times. The cells are subjected to an SW41 three tier CsCl gradient for 2 hours and a band migrating between the helper adenovirus and incomplete virus is detected.

Fractions are assayed on a 6 well plate containing 293 cells infected with 5λ of fraction for 16-20 hours in DMEM + 2% FBS. Cells are collected, washed with phosphate buffered saline, and resuspended in 2 ml PBS. 200λ of the 2ml cell fractions is cytospun onto a slide.

The cells were subjected to immunofluorescence for dystrophin as follows. Cells were fixed in 10N MeOH at -20°C. The cells were exposed to a monoclonal antibody specific for the carboxy terminus of human dystrophin [NCL-DYS2; Novocastra Laboratories Ltd., UK]. Cells were then washed three times and exposed to a secondary antibody, i.e. 1:200 goat anti-mouse IgG in FITC.

The titer/fraction for seven fractions revealed in the immunofluorescent stains were calculated by the following formula and reported in Table 2 below. DFU/field = (DFU/200λ cells) x 10 = DFU/10^6 cells = (DFU/5λ viral fraction) x 20 = DFU/100λ fraction.

Table 2

Fraction	DFU/100λ
1	--
2	--
3	6×10^3
4	1.8×10^4
5	9.6×10^3
6	200
7	200

A virus capable of transducing the dystrophin minigene is detected as a "positive" (i.e., green fluorescent) cell. The results of the IF illustrate that heat-treated fractions do not show positive immunofluorescence. Southern blot data suggest one species on the same size as the input DNA, with helper virus contamination.

The recombinant virus can be subsequently separated from the majority of helper virus by sedimentation through cesium gradients. Initial studies demonstrate that the functional AdCMVΔmDys virions are produced, but are contaminated with helper virus. Successful purification would render AdΔ virions that are incapable of encoding viral proteins but are capable of transducing murine skeletal muscle.

Example 10 - Pseudotyping

The following experiment provides a method for preparing a recombinant AdΔ according to the invention, utilizing helper viruses from serotypes which differ from that of the pAdΔ in the transfection/infection protocol. It is unexpected that the ITRs and packaging sequence of

Ad5 could be incorporated into a virion of another serotype.

A. Protocol

The basic approach is to transfect the AdΔ.CMV*lacZ* recombinant virus (Ad5) into 293 cells and subsequently infect the cell with the helper virus derived from a variety of Ad serotypes (2, 3, 4, 5, 7, 8, 12, and 40). When CPE is achieved, the lysate is harvested and banded through two cesium gradients.

More particularly, the Ad5-based plasmid pAdΔ.CMV*lacZ* of Example 1 was linearized with EcoRI. The linearized plasmids were then transfected into ten 150 mm dishes of 293 cells using calcium phosphate co-precipitation. At 10-15 hours post transfection, wild type adenoviruses (of one of the following serotypes: 2, 3, 4, 5, 7, 12, 40) were used to infect cells at an MOI of 5. The cells were then harvested at full CPE and lysed by three rounds of freeze-thawing. Pellet is resuspended in 4 mL Tris-HCl. Cell debris was removed by centrifugation and partial purification of Ad5Δ.CMV*lacZ* from helper virus was achieved with 2 rounds of CsCl gradient centrifugation (SW41 column, 35,000 rpm, 2 hours). Fractions were collected from the bottom of the tube (fraction #1) and analysed for *lacZ* transducing viruses on 293 target cells by histochemical staining (at 20h PI). Contaminating helper viruses were quantitated by plaque assay.

Except for adenovirus type 3, infection with Ad serotypes 2, 4, 5, 7, 12 and 40 were able to produce *lacZ* transducing viruses. The peak of β-galactosidase activity was detected between the two major A_{260} absorbing peaks, where most of the helper viruses banded (data not shown). The quantity of *lacZ* virus recovered from 10 plates ranged from 10^4 to 10^8 transducing particles depending on the serotype of the helper. As

expected Ad2 and Ad5 produced the highest titer of *lacZ* transducing viruses (Table 3). Wild type contamination was in general 10^2-10^3 log higher than corresponding *lacZ* titer except in the case of Ad40.

B. Results

Table 3 summarizes the growth characteristics of the wild type adenoviruses as evaluated on propagation in 293 cells. This demonstrated the feasibility of utilizing these helper viruses to infect the cell line which has been transfected with the Ad5 deleted virus.

Table 3

Adenovirus serotypes	p/ml	pfu/ml	p:pfu
2	5×10^{12}	2.5×10^{11}	20:01
3	1×10^{12}	6.25×10^{9}	160:1
4	3×10^{12}	2×10^{9}	150:1
5	1×10^{12}	5×10^{10}	20:01
7a	5×10^{12}	1×10^{11}	50:1
12	6×10^{11}	4×10^{9}	150:1
35	1.2×10^{12}		
40	2.2×10^{12}	4.4×10^{8}	5000:1

Table 4 summarizes the results of the final purified fractions. The middle column, labeled LFU/μl quantifies the production of *lacZ* forming units, which is a direct measure of the packaging and propagation of pseudotyped recombinant AdΔ virus. The pfu/μl titer is an estimate of the contaminating wild type virus. AdΔ virus pseudotyped with all adenoviral strains was generated except for Ad3. The titers range between 10^7 - 10^4.

Table 4

Serotypes	LFU/ml	PFU/ml
2	4.6×10^7	1.8×10^9
3	0	NA
4	6.7×10^6	9.3×10^7
5	6.3×10^7	1.9×10^9
7a	3×10^6	1.8×10^8
12	1.2×10^5	3.3×10^8
40	9.5×10^4	1.5×10^3

Table 5A-5D represents a more detailed analysis of the fractions from the second purification for each of the experiments summarized in Table 4. Again, LFU/μl is the recovery of the AdΔ viruses, whereas pfu/μl represents recovery of the helper virus.

Table 5A

Ad2 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	120	9532	8×10^6
2	100	5.8×10^4	3×10^6
3	100	8.24×10^4	6×10^5
4	100	9.47×10^4	1.2×10^5
5	100	6×10^4	8×10^4
6	100	2×10^4	6×10^4
7	100	5434	5×10^4
Total/10 pH		3.32×10^7	1.35×10^9

Table 5B

Ad4 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	100	1000	1.75×10^5
2	100	1.79×10^4	2.8×10^5
3	100	1.8×10^4	5.5×10^4
4	100	2909	1.25×10^4
5	100	920	4×10^4
6	100	153	3×10^3
Total/10 pH		4×10^6	5.6×10^7
Ad5 Fraction #			
1	120	1.98×10^4	6×10^6
2	100	5.8×10^4	3×10^6
3	100	1.2×10^5	1.5×10^6
4	100	1×10^5	1.4×10^5
5	100	7.96×10^4	8×10^4
6	100	6860	6×10^4
Total/10 pH		3.88×10^7	1.2×10^9

Table 5C

Ad7 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	100	1225	5×10^5
2	100	5550	4×10^5
3	100	4938	2×10^5
4	100	3866	8×10^4
5	100	4134	6×10^4
6	100	995	7×10^4
7	100	230	6×10^3
Total/10 pH		2.09×10^6	1.3×10^8
Ad12 Fraction #			
1	100	31	5×10^5
2	80	169	8.5×10^5
3	80	245	1.8×10^5
4	110	161	1.1×10^5
5	120	62	7×10^3
Total/10 pH		6.14×10^4	1.65×10^8

Table 5D

Ad40 Fraction #	VOLUME/ul	LFU/ul	PFU/ul
1	80	61	5
2	80	184	3
3	80	199	3
4	80	168	1
5	80	122	
6	100	46	
7	100	32	
Total/10 pH		6.65×10^4	1.1×10^3

C. Characterization of the Structure of Packaged Viruses

Aliquots of serial fractions were analysed by Southern blots using *lacZ* as a probe. In the case of Ad2 and 5, not only the linearized monomer was packaged but multiple forms of recombinant virus with distinct sizes were found. These forms correlated well with the sizes of dimers, trimers and other higher molecular weight concatamers. The linearized monomers peaked closer to the top of tube (the defective adenovirus band) than other forms. When these forms were correlated with *lacZ* activity, a better correlation was found between the higher molecular weight forms than the monomers. With pseudotyping of Ad4 and Ad7, no linearized monomers were packaged and only higher molecular weight forms were found.

These data definitively demonstrate the production and characterization of the Δ virus and the different pseudotypes. This example illustrates a very simple way of generating pseudotype viruses.

Example 11 - AdΔ Vector Containing a FH Gene

Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by abnormalities (deficiencies) in the function or expression of LDL receptors [M.S. Brown and J.L. Goldstein, Science, 232(4746):34-37 (1986); J.L. Goldstein and M.S. Brown, "Familial hypercholesterolemia" in Metabolic Basis of Inherited Disease., ed. C.R. Scriver et al, McGraw Hill, New York, pp1215-1250 (1989).] Patients who inherit one abnormal allele have moderate elevations in plasma LDL and suffer premature life-threatening coronary artery disease (CAD). Homozygous patients have severe hypercholesterolemia and life-threatening CAD in childhood. An FH-containing vector of the invention is constructed by replacing the lacZ minigene in the pAdΔc.CMVlacZ vector with a minigene containing the LDL receptor gene [T. Yamamoto et al, Cell, 39:27-38 (1984)] using known techniques and as described analogously for the dystrophin gene and CFTR in the preceding examples. Vectors bearing the LDL receptor gene can be readily constructed according to this invention. The resulting plasmid is termed pAdΔc.CMV-LDL.

This plasmid is useful in gene therapy of FH alone, or preferably, in the form of a conjugate prepared as described herein to substitute a normal LDL gene for the abnormal allele responsible for the gene.

A. Ex Vivo Gene Therapy

Ex vivo gene therapy can be performed by harvesting and establishing a primary culture of hepatocytes from a patient. Known techniques may be used to isolate and transduce the hepatocytes with the above vector(s) bearing the LDL receptor gene(s). For example, techniques of collagenase perfusion developed for rabbit liver can be adapted for human tissue and used in transduction. Following transduction, the hepatocytes

are removed from the tissue culture plates and reinfused into the patient using known techniques, e.g. via a catheter placed into the inferior mesenteric vein.

B. In Vivo Gene Therapy

Desirably, the *in vivo* approach to gene therapy, e.g. liver-directed, involves the use of the vectors and vector conjugates described above. A preferred treatment involves infusing a vector LDL conjugate of this invention into the peripheral circulation of the patient. The patient is then evaluated for change in serum lipids and liver tissues.

The virus or conjugate can be used to infect hepatocytes *in vivo* by direct injection into a peripheral or portal vein (10^7-10^8 pfu/kg) or retrograde into the biliary tract (same dose). This effects gene transfer into the majority of hepatocytes.

Treatments are repeated as necessary, e.g. weekly. Administration of a dose of virus equivalent to an MOI of approximately 20 (i.e. 20 pfu/hepatocyte) is anticipated to lead to high level gene expression in the majority of hepatocytes.

All references recited above are incorporated herein by reference. Numerous modifications and variations of the present invention are included in the above-identified specification and are expected to be obvious to one of skill in the art. Such modifications and alternations to the compositions and processes of the present invention, such as various modifications to the PAC sequences or the shuttle vectors, or to other sequences of the vector, helper virus and minigene components, are believed to be encompassed in the scope of the claims appended hereto.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: Improved Adenovirus and Methods of Use Thereof

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Howson and Howson
(B) STREET: Spring House Corporate Cntr, PO Box 457
(C) CITY: Spring House
(D) STATE: Pennsylvania
(E) COUNTRY: USA
(F) ZIP: 19477

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/331,381
(B) FILING DATE: 28-OCT-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Bak, Mary E.
(B) REGISTRATION NUMBER: 31,215
(C) REFERENCE/DOCKET NUMBER: GNVPN.008PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 215-540-9200
(B) TELEFAX: 215-540-5818

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7897 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TTCTGGGCGT AGGTTCGCGT GCGGTTTTCT GGGTGTTTTT TGTGGACTTT 300

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7852 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GAATTCGCTA GCTAGCGGGG GAATACATAC CCGCAGGCGT AGAGACAACA 50

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GGTAGATGAC GACCATCAGG GACAGCTTCA AGGATCGCTC GCGGCTCTTA 5600

CCAGCCTAAC TTCGATCACT GGACCGCTGA TCGTCACGGC GATTTATGCC 5650

GCCTCGGCGA GCACATGGAA CGGGTTGGCA TGGATTGTAG GCGCCGCCCT 5700

ATACCTTGTC TGCCTCCCCG CGTTGCGTCG CGGTGCATGG AGCCGGGCCA 5750

CCTCGACCTG AATGGAAGCC GGCGGCACCT CGCTAACGGA TTCACCACTC 5800

CAAGAATTGG AGCCAATCAA TTCTTGCGGA GAACTGTGAA TGCGCAAACC 5850

AACCCTTGGC AGAACATATC CATCGCGTCC GCCATCTCCA GCAGCCGCAC 5900

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CAAAACGTCT GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTG 6100

TTTCGTAAAG TCTGGAAACG CGGAAGTCAG CGCCCTGCAC CATTATGTTC 6150

CGGATCTGCA TCGCAGGATG CTGCTGGCTA CCCTGTGGAA CACCTACATC 6200

TGTATTAACG AAGCCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG 6250

GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA 6300

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TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC 6400

AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA 6450

CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC 6500

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CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT 7600

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GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG 7750

CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT 7800

CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC 7850

AA 7852

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9972 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTCG TTCGGCTGCG 50

GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA TCCACAGAAT 100

CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC 150

AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC 200

CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC 250

CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG 300

CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT 350

CCCTTCGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA 400

GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC 450

GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA 500

CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA 550

TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG 600

CCTAACTACG GCTACACTAG AAGAACAGTA TTTGGTATCT GCGCTCTGCT 650

GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC 700

AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG 750

CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC 800

TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT 850

TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT 900

AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG 950

CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA 1000

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CCATCTGGCC CCAGTGCTGC AATGATACCG CCAGACCCAC GCTCACCGGC 1100

TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA 1150

GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG 1200

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TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA 1400

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CGGAGACGGT CACAGCTTGT CTGTAAGCGG ATGCCGGGAG CAGACAAGCC 2100

CGTCAGGGCG CGTCAGCGGG TGTTGGCGGG TGTCGGGGCT GGCTTAACTA 2150

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GTGCCGTAAA GCACTAAATC GGAACCCTAA AGGGAGCCCC CGATTTAGAG 2500

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GGCCACTAGT ACTTCTCGAG CTCTGTACAT GTCCGCGGTC GCGACGTACG 2950

CGTATCGATG GCGCCAGCTG CAGGCGGCCG CCATATGCAT CCTAGGCCTA 3000

TTAATATTCC GGAGTATACG TAGCCGGCTA ACGTTAACAA CCGGTACCTC 3050

TAGAACTATA GCTAGCCAAT TCCATCATCA ATAATATACC TTATTTTGGA 3100

TTGAAGCCAA TATGATAATG AGGGGGTGGA GTTTGTGACG TGGCGCGGGG 3150

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TCTCCTCTTT CAGAGCAGCA ATCTGGGGCT TAGACTTGCA CTTGCTTGAG 4200

TTCCGGTGGG GAAAGAGCTT CACCCTGTCG GAGGGGCTGA TGGCTTGCCG 4250

GAAGAGGCTC CTCTCGTTCA GCAGTTTCTG GATGGAATCG TACTGCCGCA 4300

CTTTGTTCTC TTCTATGACC AAAAATTGTT GGCATTCCAG CATTGCTTCT 4350

ATCCTGTGTT CACAGAGAAT TACTGTGCAA TCAGCAAATG CTTGTTTTAG 4400

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GGACAGCCTT CTCTCTAAAG GCTCATCAGA ATCCTCTTCG ATGCCATTCA 6400

TTTGTAAGGG AGTCTTTTGC ACAATGGAAA ATTTTCGTAT AGAGTTGATT 6450

GGATTGAGAA TAGAATTCTT CCTTTTTTCC CCAAACTCTC CAGTCTGTTT 6500

AAAAGATTGT TTTTTTGTTT CTGTCCAGGA GACAGGAGCA TCTCCTTCTA 6550

ATGAGAAACG GTGTAAGGTC TCAGTTAGGA TTGAATTTCT TCTTTCTGCA 6600

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ACCAAGAAGT GAGAAATTAC TGAAGAAGAG GCTGTCATCA CCATTAGAAG 7300

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CCCTCCTCCC AGAAGGCTGT TACATTCTCC ATCACTACTT CTGTAGTCGT 7400

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TTTTGTTTAT TGCTCCAAGA GAGTCATACC ATGTTTGTAC AGCCCAGGGA 7500

AATTGCCGAG TGACCGCCAT GCGCAGAACA ATGCAGAATG AGATGGTGGT 7550

GAATATTTTC CGGAGGATGA TTCCTTTGAT TAGTGCATAG GGAAGCACAG 7600

ATAAAAACAC CACAAAGAAC CCTGAGAAGA AGAAGGCTGA GCTATTGAAG 7650

TATCTCACAT AGGCTGCCTT CCGAGTCAGT TTCAGTTCTG TTTGTCTTAA 7700

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TCGCCCCGCC CCGCCCCTCG CCCCGCCCCG CCCCGCCTGG CGCGCGCCCC 8850

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ACGTGGCCTC GAGTAGATGT ACTGCCAAGT AGGAAAGTCC CATAAGGTCA 9000

TGTACTGGGC ATAATGCCAG GCGGGCCATT TACCGTCATT GACGTCAATA 9050

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GCTGATCTCC CTAGACAAAT ATTACGCGCT ATGAGTAACA CAAAATTATT 9300

CAGATTTCAC TTCCTCTTAT TCAGTTTTCC CGCGAAAATG GCCAAATCTT 9350

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TATTGATGAT GCTAGCATGC GCAAATTTAA AGCGCTGATA TCGATCGCGC 9650

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GTTATAATTA CCTCAGGTCG ACGTCCCATG GCCATTCGAA TTCGTAATCA 9750

TGGTCATAGC TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA 9800

CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG 9850

TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG 9900

GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG 9950

AGGCGGTTTG CGTATTGGGC GC 9972

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TAGTAAATTT GGGC 14

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

AGTAAGATTT GGCC 14

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGTGAAATCT GAAT 14

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GAATAATTTT GTGT 14

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGTAATATTT GTCT 14

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

WANWTTTG 8

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19307 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CCAATTCCAT CATCAATAAT ATACCTTATT TTGGATTGAA GCCAATATGA 50

TAATGAGGGG GTGGAGTTTG TGACGTGGCG CGGGGCGTGG GAACGGGGCG 100

GGTGACGTAG GTTTTAGGGC GGAGTAACTT GTATGTGTTG GGAATTGTAG 150

TTTTCTTAAA ATGGGAAGTT ACGTAACGTG GGAAAACGGA AGTGACGATT 200

TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT TTCTGGGCGT AGGTTCGCGT 250

GCGGTTTTCT GGGTGTTTTT TGTGGACTTT AACCGTTACG TCATTTTTTA 300

GTCCTATATA TACTCGCTCT GCACTTGGCC CTTTTTTACA CTGTGACTGA 350

TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT TTTAATAGGT TTTCTTTTTT 400

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TAACTTTCTG GTTTTTCAGT TCCTCGAGTA CCCGATCCTC TAGAGTCCGG 14900

AGGCTGGATC GGTCCCGGTG TCTTCTATGG AGGTCAAAAC AGCGTGGATG 14950

GCGTCTCCAG GCGATCTGAC GGTTCACTAA ACGAGCTCTG CTTATATAGA 15000

CCTCCCACCG TACACGCCTA CCGCCCATTT GCGTCAATGG GGCGGAGTTG 15050

TTACGACATT TTGGAAAGTC CCGTTGATTT TGGTGCCAAA ACAAACTCCC 15100

ATTGACGTCA ATGGGGTGGA GACTTGGAAA TCCCCGTGAG TCAAACCGCT 15150

ATCCACGCCC ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAATAGCG 15200

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GTACTGGGCA TAATGCCAGG CGGGCCATTT ACCGTCATTG ACGTCAATAG 15300

GGGGCGTACT TGGCATATGA TACACTTGAT GTACTGCCAA GTGGGCAGTT 15350

TACCGTAAAT ACTCCACCCA TTGACGTCAA TGGAAAGTCC CTATTGGCGT 15400

TACTATGGGA ACATACGTCA TTATTGACGT CAATGGGCGG GGGTCGTTGG 15450

GCGGTCAGCC AGGCGGGCCA TTTACCGTAA GTTATGTAAC GACCTGCAGG 15500

TCGACTCTAG AGGATCTCCC TAGACAAATA TTACGCGCTA TGAGTAACAC 15550

AAAATTATTC AGATTTCACT TCCTCTTATT CAGTTTTCCC GCGAAAATGG 15600

CCAAATCTTA CTCGGTTACG CCCAAATTTA CTACAACATC CGCCTAAAAC 15650

CGCGCGAAAA TTGTCACTTC CTGTGTACAC CGGCGCACAC CAAAAACGTC 15700

ACTTTTGCCA CATCCGTCGC TTACATGTGT TCCGCCACAC TTGCAACATC 15750

ACACTTCCGC CACACTACTA CGTCACCCGC CCCGTTCCCA CGCCCCGCGC 15800

CACGTCACAA ACTCCACCCC CTCATTATCA TATTGGCTTC AATCCAAAAT 15850

AAGGTATATT ATTGATGATG CTAGCGGGGC CCTATATATG GATCCAATTG 15900

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AATGGCGAAT GGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG 16350

TATTTCACAC CGCATACGTC AAAGCAACCA TAGTACGCGC CCTGTAGCGG 16400

CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC 16450

TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC 16500

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CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC 16700

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TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA 16800

TTTAACGCGA ATTTTAACAA AATATTAACG TTTACAATTT TATGGTGCAC 16850

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CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC 16950

GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT GTCAGAGGTT 17000

TTCACCGTCA TCACCGAAAC GCGCGAGACG AAAGGGCCTC GTGATACGCC 17050

TATTTTTATA GGTTAATGTC ATGATAATAA TGGTTTCTTA GACGTCAGGT 17100

GGCACTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA 17150

AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC 17200

TTCAATAATA TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC 17250

GCCCTTATTC CCTTTTTTGC GGCATTTTGC CTTCCTGTTT TTGCTCACCC 17300

AGAAACGCTG GTGAAAGTAA AAGATGCTGA AGATCAGTTG GGTGCACGAG 17350

TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT TGAGAGTTTT 17400

CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG 17450

TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCGCC 17500

GCATACACTA TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA 17550

AAGCATCTTA CGGATGGCAT GACAGTAAGA GAATTATGCA GTGCTGCCAT 17600

AACCATGAGT GATAACACTG CGGCCAACTT ACTTCTGACA ACGATCGGAG 17650

GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA TCATGTAACT 17700

CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA 17750

GCGTGACACC ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAACTAT 17800

TAACTGGCGA ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG 17850

ATGGAGGCGG ATAAAGTTGC AGGACCACTT CTGCGCTCGG CCCTTCCGGC 17900

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TTTACTCATA TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA 18150

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AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA 18400

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GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC 18500

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CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC 18600

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TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA 18750

AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA 18800

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CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG GCCTTTTGCT 18900

CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC 18950

CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA 19000

GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT 19050

CTCCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC 19100

CGACTGGAAA GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA 19150

CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG 19200

TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCTATGACCA 19250

TGATTACGAA TTCGAATGGC CATGGGACGT CGACCTGAGG TAATTATAAC 19300

CCGGGCC 19307

WHAT IS CLAIMED IS:

1. A recombinant shuttle vector comprising:

(a) the DNA sequences of, or corresponding to, a portion of the genome of an adenovirus which comprises DNA sequences of, or corresponding to, the adenovirus 5' and 3' inverted terminal repeats and packaging/enhancer domain necessary for replication and virion encapsidation in the absence of sequence encoding viral genes;

(b) a selected gene operatively linked to regulatory sequences directing its expression, said gene operatively linked to the DNA of (a) and capable of expression in a target cell *in vivo* or *in vitro*.

2. The vector according to claim 1 wherein said DNA sequences (a) comprise the native adenovirus 5' inverted terminal repeats and packaging sequences.

3. The vector according to claim 1 wherein said DNA sequences (a) comprise the native adenovirus 3' inverted terminal repeat sequences.

4. The vector according to claim 1 wherein said selected gene (b) is a reporter gene.

5. The vector according to claim 4 wherein said reporter gene is selected from the group consisting of the genes encoding ß-galactosidase, alkaline phosphatase and green fluorescent protein.

6. The vector according to claim 1 wherein said selected gene (b) is a therapeutic gene.

7. The vector according to claim 6 wherein said therapeutic gene is selected from the group consisting of a normal CFTR gene, a DMD Becker allele and a normal LDL gene.

8. A crippled adenovirus helper virus comprising a modified adenovirus sequence in place of native adenovirus sequence map units 0-1, which modification reduces the packaging efficiency of said virus, said virus also containing selected adenovirus genes necessary to direct a productive viral infection.

9. The helper virus according to claim 8 wherein said modified sequence comprises:

i. a fragment of adenovirus map units 0-1;

ii. a fragment of (i) containing a 5' inverted terminal repeat and between one to four selected packaging sequences,

iii. a modified fragment of (i) containing at least one PAC consensus sequence in place of at least one native PAC sequence; and

iv. a modified fragment of (ii), wherein said native PAC sequences are mutated to contain modified sequences.

10. The virus according to claim 8 wherein said modified sequence comprises Ad5 base pairs 1-269.

11. The virus according to claim 8 wherein said sequence (ii) comprises Ad5 base pairs 1-321.

12. The virus according to claim 8 wherein said helper adenovirus is conjugated to a poly-cation sequence.

13. A method for producing a recombinant adenovirus which comprises transfecting a selected host cell with

(a) a recombinant shuttle vector comprising

i. the DNA sequences of, or corresponding to, a portion of the genome of an adenovirus which comprises adenovirus 5' and 3' cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes; and

ii. a selected gene operatively linked to regulatory sequences directing its expression, said gene linked to the DNA of (a) and capable of expression in a target cell *in vivo* or *in vitro*; and

(b) a helper adenovirus comprising sufficient adenovirus gene sequences necessary for a productive viral infection, wherein said transfected host cell permits the formation of a recombinant virus comprising the DNA of (i) and (ii) in an adenoviral capsid, and

isolating and purifying the recombinant virus from said cell.

14. The method according to claim 13, wherein said helper virus is a crippled helper virus comprising a modified adenovirus sequence in place of native adenovirus sequence map units 0-1, which modification reduces the packaging efficiency of said helper virus, said helper virus also containing selected adenovirus genes necessary to direct a productive viral infection.

15. The method according to claim 13 wherein said helper adenovirus is associated with a poly-cation sequence.

16. The method according to claim 13 wherein said vector is associated with said helper adenovirus conjugate in a single particle.

17. The method according to claim 13 wherein said helper virus is an adenovirus sequence containing deletions of all or portions of the E1a and E1b genes.

18. The method according to claim 13 wherein said helper virus is an adenovirus sequence containing deletions of all or a portion of the E3 gene.

19. A recombinant adenovirus comprising

i. the DNA of, or corresponding to, a portion of the genome of an adenovirus which comprises adenovirus 5' and 3'cis-elements necessary for replication and virion encapsidation in the absence of sequence encoding viral genes;

ii. a selected gene operatively linked to regulatory sequences directing its expression, said gene linked to the DNA of (a) and capable of expression in a target cell *in vivo* or *in vitro*;

said DNA and gene encapsidated in an adenoviral capsid.

20. The virus according to claim 19 wherein said viral capsid is a capsid of an adenovirus serotype selected from the group consisting of types 2, 4, 5, 7, 12 and 40.

21. The virus according to claim 19 wherein said selected gene is a CFTR gene, a DMD gene and an LDL gene.

22. The use of a recombinant adenovirus according to claim 19 for the manufacture of a pharmaceutical composition suitable for delivering and integrating a selected gene into the chromosome of a target cell.

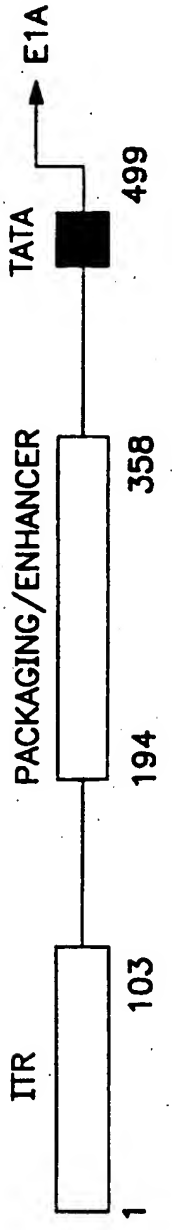
E1A
TATA
499
PACKAGING/ENHANCER
358
194
ITR
103
1

FIG. IA

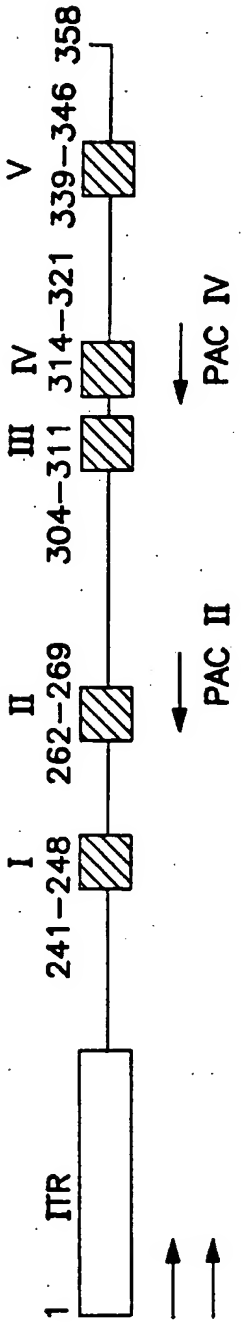
V
339–346
358
IV
314–321
PAC IV
III
304–311
II
262–269
PAC II
I
241–248
ITR
1

FIG. IB

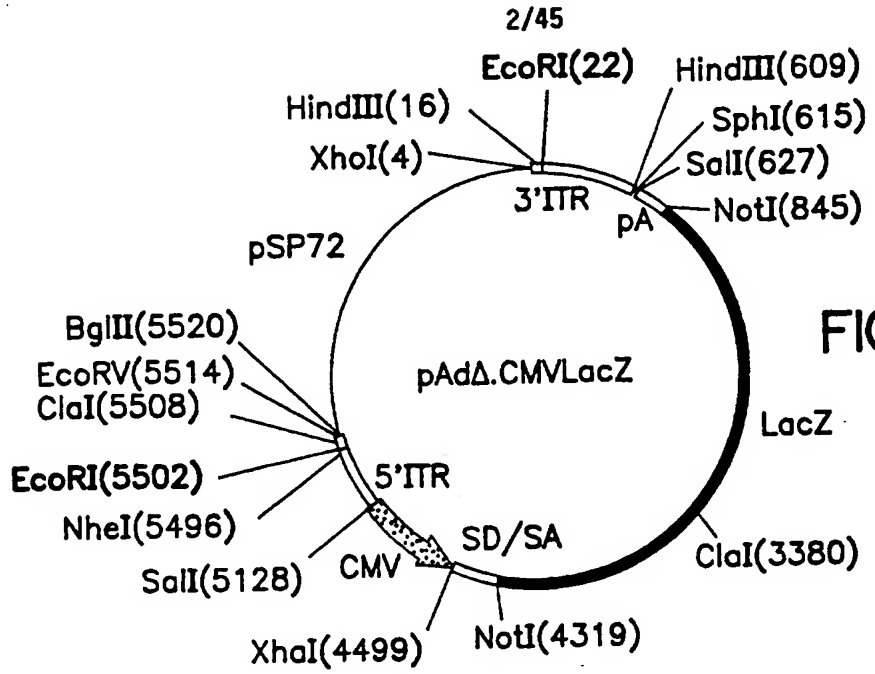

FIG. 2A

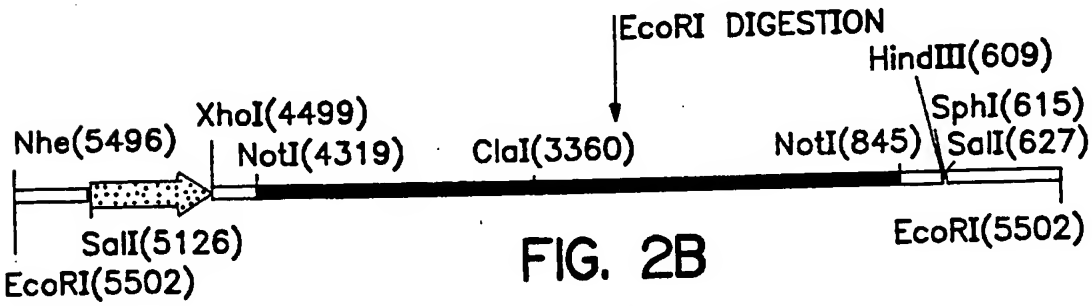

FIG. 2B

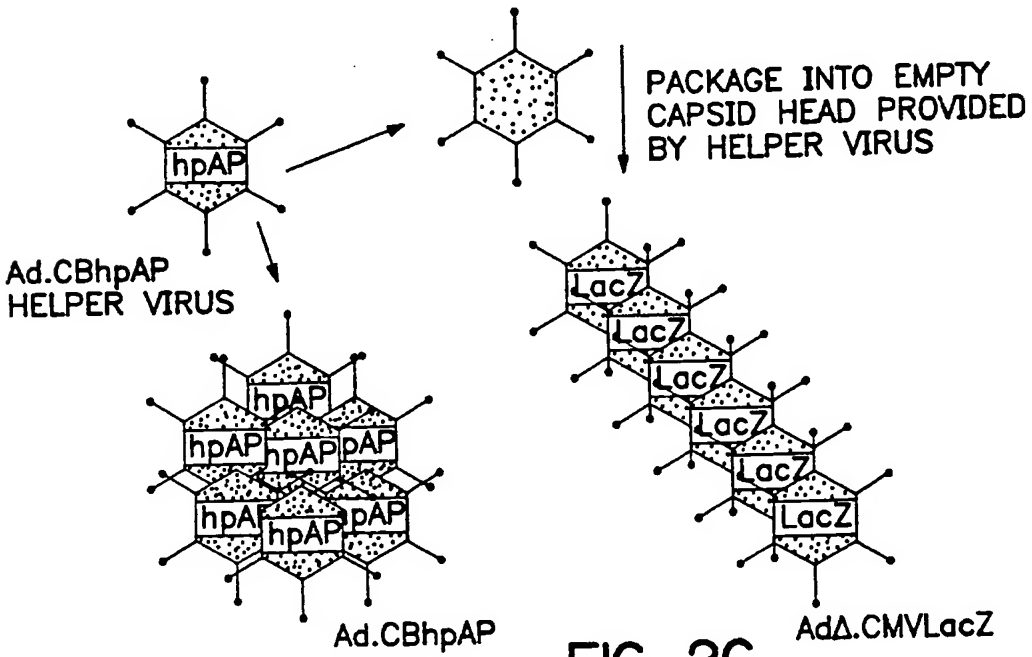

FIG. 2C

FIGURE 3A

```
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CGGGGCGTGG GAACGGGGCG GGTGACGTAG GTTTTAGGGC GGAGTAACTT     150
GTATGTGTTG GGAATTGTAG TTTTCTTAAA ATGGGAAGTT ACGTAACGTG     200
GGAAAACGGA AGTGACGATT TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT     250
TTCTGGGCGT AGGTTCGCGT GCGGTTTTCT GGGTGTTTTT TGTGGACTTT     300
AACCGTTACG TCATTTTTTA GTCCTATATA TACTCGCTCT GCACTTGGCC     350
CTTTTTTACA CTGTGACTGA TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT     400
TTTAATAGGT TTTCTTTTTT ACTGGTAAGG CTGACTGTTA GGCTGCCGCT     450
GTGAAGCGCT GTATGTTGTT CTGGAGCGGG AGGGTGCTAT TTTGCCTAGG     500
CAGGAGGGTT TTTCAGGTGT TTATGTGTTT TTCTCTCCTA TTAATTTTGT     550
TATACCTCCT ATGGGGGCTG TAATGTTGTC TCTACGCCTG CGGGTATGTA     600
TTCCCCCCAA GCTTGCATGC CTGCAGGTCG ACTCTAGAGG ATCCGAAAAA     650
ACCTCCCACA CCTCCCCCTG AACCTGAAAC ATAAAATGAA TGCAATTGTT     700
GTTGTTAACT TGTTTATTGC AGCTTATAAT GGTTACAAAT AAAGCAATAG     750
CATCACAAAT TTCACAAATA AAGCATTTTT TTCACTGCAT TCTAGTTGTG     800
GTTTGTCCAA ACTCATCAAT GTATCTTATC ATGTCTGGAT CCCCGCGGCC     850
GCCTAGAGTC GAGGCCGAGT TTGTCAGAAA GCAGACCAAA CAGCGGTTGG     900
AATAATAGCG AGAACAGAGA AATAGCGGCA AAAATAATAC CCGTATCACT     950
TTTGCTGATA TGGTTGATGT CATGTAGCCA AATCGGGAAA AACGGGAAGT    1000
AGGCTCCCAT GATAAAAAAG TAAAAGAAAA AGAATAAACC GAACATCCAA    1050
AAGTTTGTGT TTTTTAAATA GTACATAATG GATTTCCTTA CGCGAAATAC    1100
GGGCAGACAT GGCCTGCCCG GTTATTATTA TTTTTGACAC CAGACCAACT    1150
GGTAATGGTA GCGACCGGCG CTCAGCTGTA ATTCCGCCGA TACTGACGGG    1200
CTCCAGGAGT CGTCGCCACC AATCCCCATA TGGAAACCGT CGATATTCAG    1250
CCATGTGCCT TCTTCCGCGT GCAGCAGATG GCGATGGCTG CTTTCCATCA    1300
GTTGCTGTTG ACTGTAGCGG CTGATGTTGA ACTGGAAGTC GCCGCGCCAC    1350
```

FIGURE 3B

```
TGGTGTGGGC CATAATTCAA TTCGCGCGTC CCGCAGCGCA GACCGTTTTC   1400
GCTCGGGAAG ACGTACGGGG TATACATGTC TGACAATGGC AGATCCCAGC   1450
GGTCAAAACA GGCGGCAGTA AGGCGGTCGG GATAGTTTTC TTGCGGCCCT   1500
AATCCGAGCC AGTTTACCCG CTCTGCTACC TGCGCCAGCT GGCAGTTCAG   1550
GCCAATCCGC GCCGGATGCG GTGTATCGCT CGCCACTTCA ACATCAACGG   1600
TAATCGCCAT TTGACCACTA CCATCAATCC GGTAGGTTTT CCGGCTGATA   1650
AATAAGGTTT TCCCCTGATG CTGCCACGCG TGAGCGGTCG TAATCAGCAC   1700
CGCATCAGCA AGTGTATCTG CCGTGCACTG CAACAACGCT GCTTCGGCCT   1750
GGTAATGGCC CGCCGCCTTC CAGCGTTCGA CCCAGGCGTT AGGGTCAATG   1800
CGGGTCGCTT CACTTACGCC AATGTCGTTA TCCAGCGGTG CACGGGTGAA   1850
CTGATCGCGC AGCGGCGTCA GCAGTTGTTT TTTATCGCCA ATCCACATCT   1900
GTGAAAGAAA GCCTGACTGG CGGTTAAATT GCCAACGCTT ATTACCCAGC   1950
TCGATGCAAA AATCCATTTC GCTGGTGGTC AGATGCGGGA TGGCGTGGGA   2000
CGCGGCGGGG AGCGTCACAC TGAGGTTTTC CGCCAGACGC CACTGCTGCC   2050
AGGCGCTGAT GTGCCCGGCT TCTGACCATG CGGTCGCGTT CGGTTGCACT   2100
ACGCGTACTG TGAGCCAGAG TTGCCCGGCG CTCTCCGGCT GCGGTAGTTC   2150
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CACCGCTTGC CAGCGGCTTA CCATCCAGCG CCACCATCCA GTGCAGGAGC   2250
TCGTTATCGC TATGACGGAA CAGGTATTCG CTGGTCACTT CGATGGTTTG   2300
CCCGGATAAA CGGAACTGGA AAAACTGCTG CTGGTGTTTT GCTTCCGTCA   2350
GCGCTGGATG CGGCGTGCGG TCGGCAAAGA CCAGACCGTT CATACAGAAC   2400
TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCACGG   2450
GTTGCCGTTT TCATCATATT TAATCAGCGA CTGATCCACC CAGTCCCAGA   2500
CGAAGCCGCC CTGTAAACGG GGATACTGAC GAAACGCCTG CCAGTATTTA   2550
GCGAAACCGC CAAGACTGTT ACCCATCGCG TGGGCGTATT CGCAAAGGAT   2600
CAGCGGGCGC GTCTCTCCAG GTAGCGAAAG CCATTTTTTG ATGGACCATT   2650
```

FIGURE 3C

TCGGCACAGC CGGGAAGGGC TGGTCTTCAT CCACGCGCGC GTACATCGGG 2700

CAAATAATAT CGGTGGCCGT GGTGTCGGCT CCGCCGCCTT CATACTGCAC 2750

CGGGCGGGAA GGATCGACAG ATTTGATCCA GCGATACAGC GCGTCGTGAT 2800

TAGCGCCGTG GCCTGATTCA TTCCCCAGCG ACCAGATGAT CACACTCGGG 2850

TGATTACGAT CGCGCTGCAC CATTCGCGTT ACGCGTTCGC TCATCGCCGG 2900

TAGCCAGCGC GGATCATCGG TCAGACGATT CATTGGCACC ATGCCGTGGG 2950

TTTCAATATT GGCTTCATCC ACCACATACA GGCCGTAGCG GTCGCACAGC 3000

GTGTACCACA GCGGATGGTT CGGATAATGC GAACAGCGCA CGGCGTTAAA 3050

GTTGTTCTGC TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA 3100

TGACCTGACC ATGCAGAGGA TGATGCTCGT GACGGTTAAC GCCTCGAATC 3150

AGCAACGGCT TGCCGTTCAG CAGCAGCAGA CCATTTTCAA TCCGCACCTC 3200

GCGGAAACCG ACATCGCAGG CTTCTGCTTC AATCAGCGTG CCGTCGGCGG 3250

TGTGCAGTTC AACCACCGCA CGATAGAGAT TCGGGATTTC GGCGCTCCAC 3300

AGTTTCGGGT TTTCGACGTT CAGACGTAGT GTGACGCGAT CGGCATAACC 3350

ACCACGCTCA TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA 3400

CCTGCGTTTC ACCCTGCCAT AAAGAAACTG TTACCCGTAG GTAGTCACGC 3450

AACTCGCCGC ACATCTGAAC TTCAGCCTCC AGTACAGCGC GGCTGAAATC 3500

ATCATTAAAG CGAGTGGCAA CATGGAAATC GCTGATTTGT GTAGTCGGTT 3550

TATGCAGCAA CGAGACGTCA CGGAAAATGC CGCTCATCCG CCACATATCC 3600

TGATCTTCCA GATAACTGCC GTCACTCCAA CGCAGCACCA TCACCGCGAG 3650

GCGGTTTTCT CCGGCGCGTA AAAATGCGCT CAGGTCAAAT TCAGACGGCA 3700

AACGACTGTC CTGGCCGTAA CCGACCCAGC GCCCGTTGCA CCACAGATGA 3750

AACGCCGAGT TAACGCCATC AAAAATAATT CGCGTCTGGC CTTCCTGTAG 3800

CCAGCTTTCA TCAACATTAA ATGTGAGCGA GTAACAACCC GTCGGATTCT 3850

CCGTGGGAAC AAACGGCGGA TTGACCGTAA TGGGATAGGT TACGTTGGTG 3900

TAGATGGGCG CATCGTAACC GTGCATCTGC CAGTTTGAGG GGACGACGAC 3950

FIGURE 3D

AGTATCGGCC TCAGGAAGAT CGCACTCCAG CCAGCTTTCC GGCACCGCTT 4000

CTGGTGCCGG AAACCAGGCA AAGCGCCATT CGCCATTCAG GCTGCGCAAC 4050

TGTTGGGAAG GGCGATCGGT GCGGGCCTCT TCGCTATTAC GCCAGCTGGC 4100

CAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG CCAGGGTTTT 4150

CCCAGTCACG ACGTTGTAAA ACGACGGGAT CGCGCTTGAG CAGCTCCTTG 4200

CTGGTGTCCA GACCAATGCC TCCCAGACCG GCAACGAAAA TCACGTTCTT 4250

GTTGGTCAAA GTAAACGACA TGGTGACTTC TTTTTTGCTT TAGCAGGCTC 4300

TTTCGATCCC CGGGAATTGC GGCCGCGGGT ACAATTCCGC AGCTTTTAGA 4350

GCAGAAGTAA CACTTCCGTA CAGGCCTAGA AGTAAAGGCA ACATCCACTG 4400

AGGAGCAGTT CTTTGATTTG CACCACCACC GGATCCGGGA CCTGAAATAA 4450

AAGACAAAAA GACTAAACTT ACCAGTTAAC TTTCTGGTTT TTCAGTTCCT 4500

CGAGTACCGG ATCCTCTAGA GTCCGGAGGC TGGATCGGTC CCGGTCTCTT 4550

CTATGGAGGT CAAAACAGCG TGGATGGCGT CTCCAGGCGA TCTGACGGTT 4600

CACTAAACGA GCTCTGCTTA TATAGACCTC CCACCGTACA CGCCTACCGC 4650

CCATTTGCGT CAATGGGGCG GAGTTGTTAC GACATTTTGG AAAGTCCCGT 4700

TGATTTTGGT GCCAAAACAA ACTCCCATTG ACGTCAATGG GGTGGAGACT 4750

TGGAAATCCC CGTGAGTCAA ACCGCTATCC ACGCCCATTG ATGTACTGCC 4800

AAAACCGCAT CACCATGGTA ATAGCGATGA CTAATACGTA GATGTACTGC 4850

CAAGTAGGAA AGTCCCATAA GGTCATGTAC TGGGCATAAT GCCAGGCGGG 4900

CCATTTACCG TCATTGACGT CAATAGGGGG CGTACTTGGC ATATGATACA 4950

CTTGATGTAC TGCCAAGTGG GCAGTTTACC GTAAATACTC CACCCATTGA 5000

CGTCAATGGA AAGTCCCTAT TGGCGTTACT ATGGGAACAT ACGTCATTAT 5050

TGACGTCAAT GGGCGGGGGT CGTTGGGCGG TCAGCCAGGC GGGCCATTTA 5100

CCGTAAGTTA TGTAACGACC TGCAGGTCGA CTCTAGAGGA TCTCCCTAGA 5150

CAAATATTAC GCGCTATGAG TAACACAAAA TTATTCAGAT TTCACTTCCT 5200

CTTATTCAGT TTTCCCGCGA AAATGGCCAA ATCTTACTCG GTTACGCCCA 5250

FIGURE 3E

```
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ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC CACCCCCTCA   5450
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CGAATTCATC GATGATATCA GATCTGCCGG TCTCCCTATA GTGAGTCGTA   5550
TTAATTTCGA TAAGCCAGGT TAACCTGCAT TAATGAATCG GCCAACGCGC   5600
GGGGAGAGGC GGTTTGCGTA TTGGGCGCTC TTCCGCTTCC TCGCTCACTG   5650
ACTCGCTGCG CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA   5700
AAGGCGGTAA TACGGTTATC CACAGAATCA GGGGATAACG CAGGAAAGAA   5750
CATGTGAGCA AAAGGCCAGC AAAAGGCCAG GAACCGTAAA AAGGCCGCGT   5800
TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA TCACAAAAAT   5850
CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA   5900
GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC   5950
CGCTTACCGG ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT   6000
TCTCAATGCT CACGCTGTAG GTATCTCAGT TCGGTGTAGG TCGTTCGCTC   6050
CAAGCTGGGC TGTGTGCACG AACCCCCCGT TCAGCCCGAC CGCTGCGCCT   6100
TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA CGACTTATCG   6150
CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG   6200
CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA   6250
GGACAGTATT TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA   6300
AGAGTTGGTA GCTCTTGATC CGGCAAACAA ACCACCGCTG CTAGCGGTGG   6350
TTTTTTTGTT TGCAAGCAGC AGATTACGCG CAGAAAAAAA GGATCTCAAG   6400
AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG GAACGAAAAC   6450
TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA TCTTCACCTA   6500
GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA AGTATATATG   6550
```

FIGURE 3F

AGTAAACTTG GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC 6600

TCAGCGATCT GTCTATTTCG TTCATCCATA GTTGCCTGAC TCCCCGTCGT 6650

GTAGATAACT ACGATACGGG AGGGCTTACC ATCTGGCCCC AGTGCTGCAA 6700

TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC AGCAATAAAC 6750

CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA CTTTATCCGC 6800

CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC 6850

CAGTTAATAG TTTGCGCAAC GTTGTTGCCA TTGCTACAGG CATCGTGGTG 6900

TCACGCTCGT CGTTTGGTAT GGCTTCATTC AGCTCCGGTT CCCAACGATC 6950

AAGGCGAGTT ACATGATCCC CCATGTTGTG CAAAAAAGCG GTTAGCTCCT 7000

TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT GTTATCACTC 7050

ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG 7100

ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT 7150

GTATGCGGCG ACCGAGTTGC TCTTGCCCGG CGTCAATACG GGATAATACC 7200

GCGCCACATA GCAGAACTTT AAAAGTGCTC ATCATTGGAA AACGTTCTTC 7250

GGGGCGAAAA CTCTCAAGGA TCTTACCGCT GTTGAGATCC AGTTCGATGT 7300

AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC TTTCACCAGC 7350

GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT 7400

AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT 7450

ATTGAAGCAT TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA 7500

TGTATTTAGA AAAATAAACA AATAGGGGTT CCGCGCACAT TTCCCCGAAA 7550

AGTGCCACCT GACGTCTAAG AAACCATTAT TATCATGACA TTAACCTATA 7600

AAAATAGGCG TATCACGAGG CCCTTTCGTC TCGCGCGTTT CGGTGATGAC 7650

GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA CAGCTTGTCT 7700

GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG 7750

TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA 7800

CTGAGAGTGC ACCATATGGA CATATTGTCG TTAGAACGCG GCTACAATTA 7850

ATACATAACC TTATGTATCA TACACATACG ATTTAGGTGA CACTATA 7897

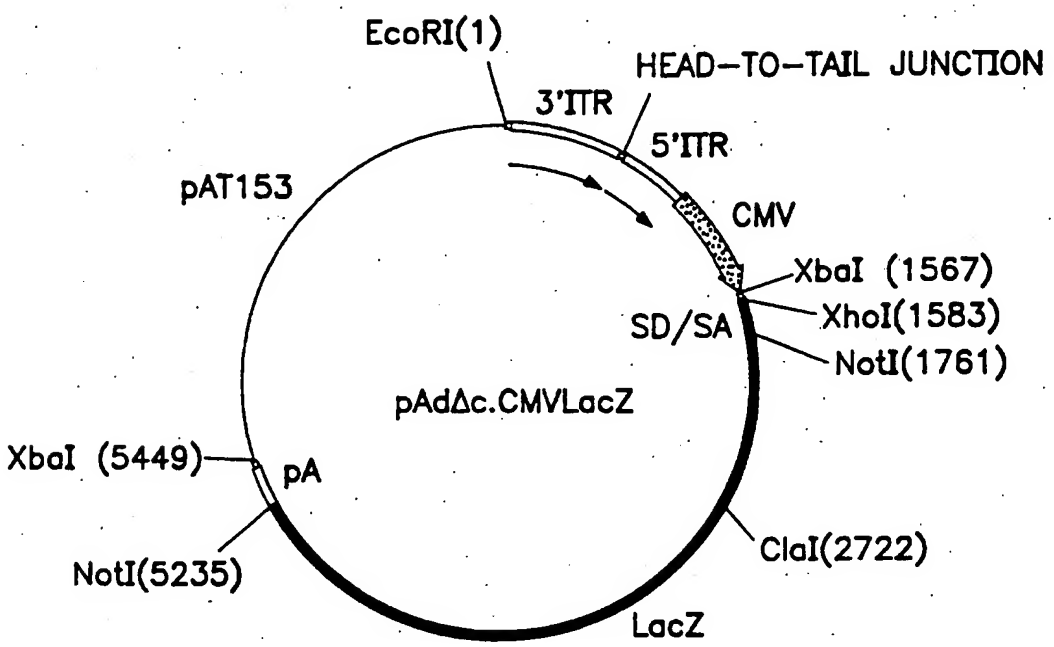
EcoRI(1)
HEAD–TO–TAIL JUNCTION
3'ITR
5'ITR
pAT153
CMV
XbaI (1567)
XhoI(1583)
SD/SA
NotI(1761)
pAdΔc.CMVLacZ
XbaI (5449)
pA
NotI(5235)
ClaI(2722)
LacZ

FIG. 4A

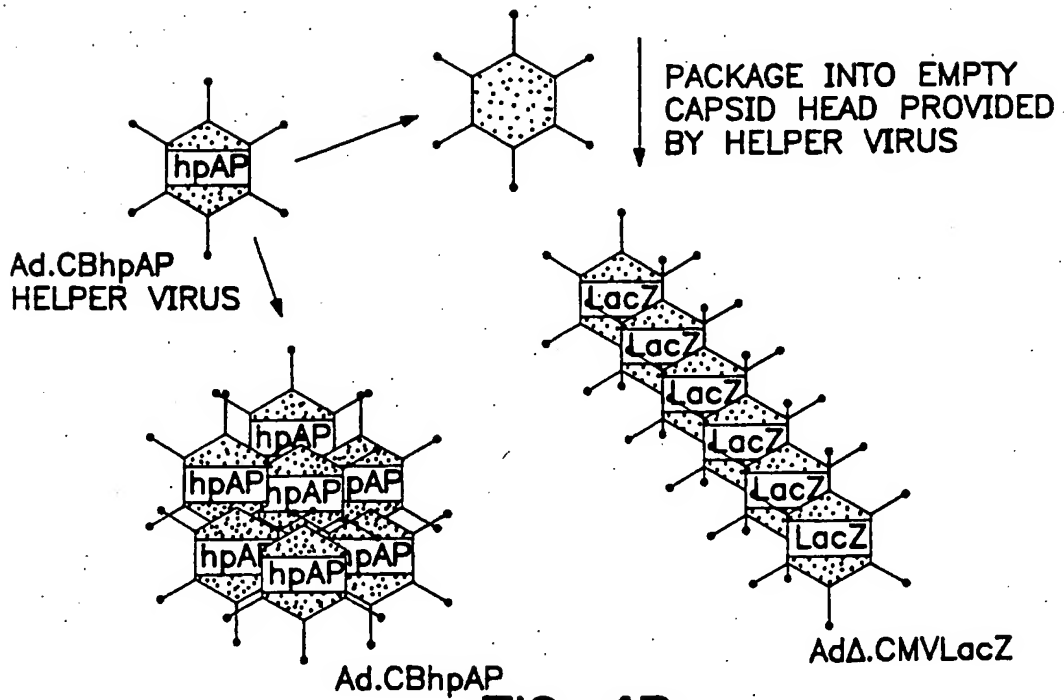
PACKAGE INTO EMPTY CAPSID HEAD PROVIDED BY HELPER VIRUS
hpAP
Ad.CBhpAP HELPER VIRUS
LacZ
hpAP
pAP
Ad.CBhpAP
AdΔ.CMVLacZ

FIG. 4B

FIGURE 5A

```
GAATTCGCTA GCTAGCGGGG GAATACATAC CCGCAGGCGT AGAGACAACA      50
TTACAGCCCC CATAGGAGGT ATAACAAAAT TAATAGGAGA GAAAAACACA     100
TAAACACCTG AAAAACCCTC CTGCCTAGGC AAAATAGCAC CCTCCCGCTC     150
CAGAACAACA TACAGCGCTT CACAGCGGCA GCCTAACAGT CAGCCTTACC     200
AGTAAAAAAG AAAACCTATT AAAAAAACAC CACTCGACAC GGCACCAGCT     250
CAATCAGTCA CAGTGTAAAA AAGGGCCAAG TGCAGAGCGA GTATATATAG     300
GACTAAAAAA TGACGTAACG GTTAAAGTCC ACAAAAAACA CCCAGAAAAC     350
CGCACGCGAA CCTACGCCCA GAAACGAAAG CCAAAAAACC CACAACTTCC     400
TCAAATCGTC ACTTCCGTTT TCCCACGTTA CGTAACTTCC CATTTTAAGA     450
AAACTACAAT TCCCAACACA TACAAGTTAC TCCGCCCTAA AACCTACGTC     500
ACCCGCCCCG TTCCCACGCC CCGCGCCACG TCACAAACTC CACCCCCTCA     550
TTATCATATT GGCTTCAATC CAAAATAAGG TATATTATTG ATGATGCTAG     600
CATCATCAAT AATATACCTT ATTTTGGATT GAAGCCAATA TGATAATGAG     650
GGGGTGGAGT TTGTGACGTG GCGCGGGGCG TGGGAACGGG GCGGGTGACG     700
TAGTAGTGTG GCGGAAGTGT GATGTTGCAA GTGTGGCGGA ACACATGTAA     750
GCGACGGATG TGGCAAAAGT GACGTTTTTG GTGTGCGCCG GTGTACACAG     800
GAAGTGACAA TTTTCGCGCG GTTTTAGGCG GATGTTGTAG TAAATTTGGG     850
CGTAACCGAG TAAGATTTGG CCATTTTCGC GGGAAAACTG AATAAGAGGA     900
AGTGAAATCT GAATAATTTT GTGTTACTCA TAGCGCGTAA TATTTGTCTA     950
GGGAGATCAG CCTGCAGGTC GTTACATAAC TTACGGTAAA TGGCCCGCCT    1000
GGCTGACCGC CCAACGACCC CCGCCCATTG ACGTCAATAA TGACGTATGT    1050
TCCCATAGTA ACGCCAATAG GGACTTTCCA TTGACGTCAA TGGGTGGAGT    1100
ATTTACGGTA AACTGCCCAC TTGGCAGTAC ATCAAGTGTA TCATATGCCA    1150
AGTACGCCCC CTATTGACGT CAATGACGGT AAATGGCCCG CCTGGCATTA    1200
TGCCCAGTAC ATGACCTTAT GGGACTTTCC TACTTGGCAG TACATCTACG    1250
TATTAGTCAT CGCTATTACC ATGGTGATGC GGTTTTGGCA GTACATCAAT    1300
```

FIGURE 5B

GGGCGTGGAT AGCGGTTTGA CTCACGGGGA TTTCCAAGTC TCCACCCCAT 1350

TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA 1400

AATGTCGTAA CAACTCCGCC CCATTGACGC AAATGGGCGG TAGGCGTGTA 1450

CGGTGGGAGG TCTATATAAG CAGAGCTCGT TTAGTGAACC GTCAGATCGC 1500

CTGGAGACGC CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGGGACC 1550

GATCCAGCCT CCGGACTCTA GAGGATCCGG TACTCGAGGA ACTGAAAAAC 1600

CAGAAAGTTA ACTGGTAAGT TTAGTCTTTT TGTCTTTTAT TTCAGGTCCC 1650

GGATCCGGTG GTGGTGCAAA TCAAAGAACT GCTCCTCAGT GGATGTTGCC 1700

TTTACTTCTA GGCCTGTACG GAAGTGTTAC TTCTGCTCTA AAAGCTGCGG 1750

AATTGTACCC GCGGCCGCAA TTCCCGGGGA TCGAAAGAGC CTGCTAAAGC 1800

AAAAAAGAAG TCACCATGTC GTTTACTTTG ACCAACAAGA ACGTGATTTT 1850

CGTTGCCGGT CTGGGAGGCA TTGGTCTGGA CACCAGCAAG GAGCTGCTCA 1900

AGCGCGATCC CGTCGTTTTA CAACGTCGTG ACTGGGAAAA CCCTGGCGTT 1950

ACCCAACTTA ATCGCCTTGC AGCACATCCC CCTTTCGCCA GCTGGCGTAA 2000

TAGCGAAGAG GCCCGCACCG ATCGCCCTTC CCAACAGTTG CGCAGCCTGA 2050

ATGGCGAATG GCGCTTTGCC TGGTTTCCGG CACCAGAAGC GGTGCCGGAA 2100

AGCTGGCTGG AGTGCGATCT TCCTGAGGCC GATACTGTCG TCGTCCCCTC 2150

AAACTGGCAG ATGCACGGTT ACGATGCGCC CATCTACACC AACGTAACCT 2200

ATCCCATTAC GGTCAATCCG CCGTTTGTTC CCACGGAGAA TCCGACGGGT 2250

TGTTACTCGC TCACATTTAA TGTTGATGAA AGCTGGCTAC AGGAAGGCCA 2300

GACGCGAATT ATTTTTGATG GCGTTAACTC GGCGTTTCAT CTCTGGTGCA 2350

ACGGGCGCTG GGTCGGTTAC GGCCAGGACA GTCGTTTGCC GTCTGAATTT 2400

GACCTGAGCG CATTTTTACG CGCCGGAGAA AACCGCCTCG CGGTGATGGT 2450

GCTGCGTTGG AGTGACGGCA GTTATCTGGA AGATCAGGAT ATGTGGCGGA 2500

TGAGCGGCAT TTTCCGTGAC GTCTCGTTGC TGCATAAACC GACTACACAA 2550

ATCAGCGATT TCCATGTTGC CACTCGCTTT AATGATGATT TCAGCCGCGC 2600

FIGURE 5C

```
TGTACTGGAG GCTGAAGTTC AGATGTGCGG CGAGTTGCGT GACTACCTAC   2650
GGGTAACAGT TTCTTTATGG CAGGGTGAAA CGCAGGTCGC CAGCGGCACC   2700
GCGCCTTTCG GCGGTGAAAT TATCGATGAG CGTGGTGGTT ATGCCGATCG   2750
CGTCACACTA CGTCTGAACG TCGAAAACCC GAAACTGTGG AGCGCCGAAA   2800
TCCCGAATCT CTATCGTGCG GTGGTTGAAC TGCACACCGC CGACGGCACG   2850
CTGATTGAAG CAGAAGCCTG CGATGTCGGT TTCCGCGAGG TGCGGATTGA   2900
AAATGGTCTG CTGCTGCTGA ACGGCAAGCC GTTGCTGATT CGAGGCGTTA   2950
ACCGTCACGA GCATCATCCT CTGCATGGTC AGGTCATGGA TGAGCAGACC   3000
ATGGTGCAGG ATATCCTGCT GATGAAGCAG AACAACTTTA ACGCCGTGCG   3050
CTGTTCGCAT TATCCGAACC ATCCGCTGTG GTACACGCTG TGCGACCGCT   3100
ACGGCCTGTA TGTGGTGGAT GAAGCCAATA TTGAAACCCA CGGCATGGTG   3150
CCAATGAATC GTCTGACCGA TGATCCGCGC TGGCTACCGG CGATGAGCGA   3200
ACGCGTAACG CGAATGGTGC AGCGCGATCG TAATCACCCG AGTGTGATCA   3250
TCTGCTCGCT GGGGAATGAA TCAGGCCACG GCGCTAATCA CGACGCGCTG   3300
TATCGCTGGA TCAAATCTGT CGATCCTTCC CGCCCGGTGC AGTATGAAGG   3350
CGGCGGAGCC GACACCACGG CCACCGATAT TATTTGCCCG ATGTACGCGC   3400
GCGTGGATGA AGACCAGCCC TTCCCGGCTG TGCCGAAATG GTCCATCAAA   3450
AAATGGCTTT CGCTACCTGG AGAGACGCGC CCGCTGATCC TTTGCGAATA   3500
CGCCCACGCG ATGGGTAACA GTCTTGGCGG TTTCGCTAAA TACTGGCAGG   3550
CGTTTCGTCA GTATCCCCGT TTACAGGGCG GCTTCGTCTG GGACTGGGTG   3600
GATCAGTCGC TGATTAAATA TGATGAAAAC GGCAACCCGT GGTCGGCTTA   3650
CGGCGGTGAT TTTGGCGATA CGCCGAACGA TCGCCAGTTC TGTATGAACG   3700
GTCTGGTCTT TGCCGACCGC ACGCCGCATC CAGCGCTGAC GGAAGCAAAA   3750
CACCAGCAGC AGTTTTTCCA GTTCCGTTTA TCCGGGCAAA CCATCGAAGT   3800
GACCAGCGAA TACCTGTTCC GTCATAGCGA TAACGAGCTC CTGCACTGGA   3850
TGGTGGCGCT GGATGGTAAG CCGCTGGCAA GCGGTGAAGT GCCTCTGGAT   3900
```

FIGURE 5D

GTCGCTCCAC AAGGTAAACA GTTGATTGAA CTGCCTGAAC TACCGCAGCC 3950

GGAGAGCGCC GGGCAACTCT GGCTCACAGT ACGCGTAGTG CAACCGAACG 4000

CGACCGCATG GTCAGAAGCC GGGCACATCA GCGCCTGGCA GCAGTGGCGT 4050

CTGGCGGAAA ACCTCAGTGT GACGCTCCCC GCCGCGTCCC ACGCCATCCC 4100

GCATCTGACC ACCAGCGAAA TGGATTTTTG CATCGAGCTG GGTAATAAGC 4150

GTTGGCAATT TAACCGCCAG TCAGGCTTTC TTTCACAGAT GTGGATTGGC 4200

GATAAAAAAC AACTGCTGAC GCCGCTGCGC GATCAGTTCA CCCGTGCACC 4250

GCTGGATAAC GACATTGGCG TAAGTGAAGC GACCCGCATT GACCCTAACG 4300

CCTGGGTCGA ACGCTGGAAG GCGGCGGGCC ATTACCAGGC CGAAGCAGCG 4350

TTGTTGCAGT GCACGGCAGA TACACTTGCT GATGCGGTGC TGATTACGAC 4400

CGCTCACGCG TGGCAGCATC AGGGGAAAAC CTTATTTATC AGCCGGAAAA 4450

CCTACCGGAT TGATGGTAGT GGTCAAATGG CGATTACCGT TGATGTTGAA 4500

GTGGCGAGCG ATACACCGCA TCCGGCGCGG ATTGGCCTGA ACTGCCAGCT 4550

GGCGCAGGTA GCAGAGCGGG TAAACTGGCT CGGATTAGGG CCGCAAGAAA 4600

ACTATCCCGA CCGCCTTACT GCCGCCTGTT TTGACCGCTG GGATCTGCCA 4650

TTGTCAGACA TGTATACCCC GTACGTCTTC CCGAGCGAAA ACGGTCTGCG 4700

CTGCGGGACG CGCGAATTGA ATTATGGCCC ACACCAGTGG CGCGGCGACT 4750

TCCAGTTCAA CATCAGCCGC TACAGTCAAC AGCAACTGAT GGAAACCAGC 4800

CATCGCCATC TGCTGCACGC GGAAGAAGGC ACATGGCTGA ATATCGACGG 4850

TTTCCATATG GGGATTGGTG GCGACGACTC CTGGAGCCCG TCAGTATCGG 4900

CGGAATTACA GCTGAGCGCC GGTCGCTACC ATTACCAGTT GGTCTGGTGT 4950

CAAAAATAAT AATAACCGGG CAGGCCATGT CTGCCCGTAT TTCGCGTAAG 5000

GAAATCCATT ATGTACTATT TAAAAAACAC AAACTTTTGG ATGTTCGGTT 5050

TATTCTTTTT CTTTTACTTT TTTATCATGG GAGCCTACTT CCCGTTTTTC 5100

CCGATTTGGC TACATGACAT CAACCATATC AGCAAAAGTG ATACGGGTAT 5150

TATTTTTGCC GCTATTTCTC TGTTCTCGCT ATTATTCCAA CCGCTGTTTG 5200

GTCTGCTTTC TGACAAACTC GGCCTCGACT CTAGGCGGCC GCGGGGATCC 5250

FIGURE 5E

AGACATGATA AGATACATTG ATGAGTTTGG ACAAACCACA ACTAGAATGC 5300

AGTGAAAAAA ATGCTTTATT TGTGAAATTT GTGATGCTAT TGCTTTATTT 5350

GTAACCATTA TAAGCTGCAA TAAACAAGTT AACAACAACA ATTGCATTCA 5400

TTTTATGTTT CAGGTTCAGG GGGAGGTGTG GGAGGTTTTT TCGGATCCTC 5450

TAGAGTCGAC GACGCGAGGC TGGATGGCCT TCCCCATTAT GATTCTTCTC 5500

GCTTCCGGCG GCATCGGGAT GCCCGCGTTG CAGGCCATGC TGTCCAGGCA 5550

GGTAGATGAC GACCATCAGG GACAGCTTCA AGGATCGCTC GCGGCTCTTA 5600

CCAGCCTAAC TTCGATCACT GGACCGCTGA TCGTCACGGC GATTTATGCC 5650

GCCTCGGCGA GCACATGGAA CGGGTTGGCA TGGATTGTAG GCGCCGCCCT 5700

ATACCTTGTC TGCCTCCCCG CGTTGCGTCG CGGTGCATGG AGCCGGGCCA 5750

CCTCGACCTG AATGGAAGCC GGCGGCACCT CGCTAACGGA TTCACCACTC 5800

CAAGAATTGG AGCCAATCAA TTCTTGCGGA GAACTGTGAA TGCGCAAACC 5850

AACCCTTGGC AGAACATATC CATCGCGTCC GCCATCTCCA GCAGCCGCAC 5900

GCGGCGCATC TCGGGCAGCG TTGGGTCCTG GCCACGGGTG CGCATGATCG 5950

TGCTCCTGTC GTTGAGGACC CGGCTAGGCT GGCGGGGTTG CCTTACTGGT 6000

TAGCAGAATG AATCACCGAT ACGCGAGCGA ACGTGAAGCG ACTGCTGCTG 6050

CAAAACGTCT GCGACCTGAG CAACAACATG AATGGTCTTC GGTTTCCGTG 6100

TTTCGTAAAG TCTGGAAACG CGGAAGTCAG CGCCCTGCAC CATTATGTTC 6150

CGGATCTGCA TCGCAGGATG CTGCTGGCTA CCCTGTGGAA CACCTACATC 6200

TGTATTAACG AAGCCTTTCT CAATGCTCAC GCTGTAGGTA TCTCAGTTCG 6250

GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA 6300

GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG 6350

TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC 6400

AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA 6450

CTACGGCTAC ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC 6500

CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG CAAACAAACC 6550

FIGURE 5F

```
ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG   6600
AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG   6650
CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA   6700
AAAAGGATCT TCACCTAGAT CCTTTTAAAT TA\AAATGAA GTTTTAAATC   6750
AATCTAAAGT ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA   6800
TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC ATCCATAGTT   6850
GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC   6900
TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG   6950
ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT   7000
CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC   7050
TAGAGTAAGT AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG   7100
CTGCAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC TTCATTCAGC   7150
TCCGGTTCCC AACGATCAAG GCGAGTTACA TCATCCCCCA TGTTGTGCAA   7200
AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG   7250
CCGCAGTGTT ATCACTCATG GTTATGCCAG CACTGCATAA TTCTCTTACT   7300
GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA   7350
GTCATTCTGA GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT   7400
CAACACGGGA TAATACCGCG CCACATAGCA CAACTTTAAA AGTGCTCATC   7450
ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT   7500
GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT   7550
CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT   7600
GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT   7650
CTTCCTTTTT CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA   7700
GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT AGGGGTTCCG   7750
CGCACATTTC CCCGAAAAGT GCCACCTGAC GTCTAAGAAA CCATTATTAT   7800
CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC TTTCGTCTTC   7850
AA                                                      7852
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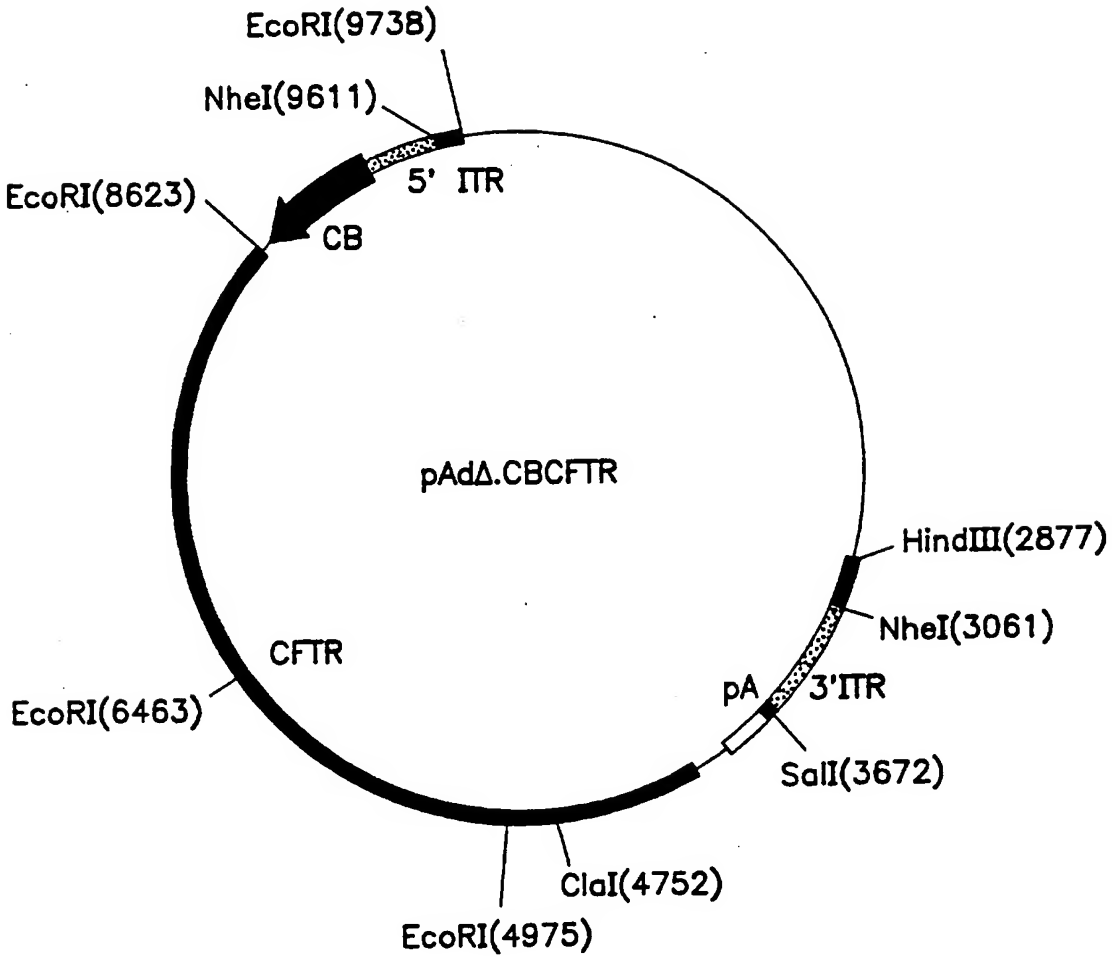

EcoRI(9738)
NheI(9611)
5' ITR
EcoRI(8623)
CB
pAdΔ.CBCFTR
HindIII(2877)
NheI(3061)
CFTR
pA
3'ITR
EcoRI(6463)
SalI(3672)
ClaI(4752)
EcoRI(4975)

FIG. 6

FIGURE 7A

```
TCTTCCGCTT CCTCGCTCAC TGACTCGCTG CGCTCGGTCG TTCGGCTGCG      50
GCGAGCGGTA TCAGCTCACT CAAAGGCGGT AATACGGTTA TCCACAGAAT     100
CAGGGGATAA CGCAGGAAAG AACATGTGAG CAAAAGGCCA GCAAAAGGCC     150
AGGAACCGTA AAAAGGCCGC GTTGCTGGCG TTTTTCCATA GGCTCCGCCC     200
CCCTGACGAG CATCACAAAA ATCGACGCTC AAGTCAGAGG TGGCGAAACC     250
CGACAGGACT ATAAAGATAC CAGGCGTTTC CCCCTGGAAG CTCCCTCGTG     300
CGCTCTCCTG TTCCGACCCT GCCGCTTACC GGATACCTGT CCGCCTTTCT     350
CCCTTCGGGA AGCGTGGCGC TTTCTCATAG CTCACGCTGT AGGTATCTCA     400
GTTCGGTGTA GGTCGTTCGC TCCAAGCTGG GCTGTGTGCA CGAACCCCCC     450
GTTCAGCCCG ACCGCTGCGC CTTATCCGGT AACTATCGTC TTGAGTCCAA     500
CCCGGTAAGA CACGACTTAT CGCCACTGGC AGCAGCCACT GGTAACAGGA     550
TTAGCAGAGC GAGGTATGTA GGCGGTGCTA CAGAGTTCTT GAAGTGGTGG     600
CCTAACTACG GCTACACTAG AAGAACAGTA TTTGGTATCT GCGCTCTGCT     650
GAAGCCAGTT ACCTTCGGAA AAAGAGTTGG TAGCTCTTGA TCCGGCAAAC     700
AAACCACCGC TGGTAGCGGT GGTTTTTTTG TTTGCAAGCA GCAGATTACG     750
CGCAGAAAAA AAGGATCTCA AGAAGATCCT TTGATCTTTT CTACGGGGTC     800
TGACGCTCAG TGGAACGAAA ACTCACGTTA AGGGATTTTG GTCATGAGAT     850
TATCAAAAAG GATCTTCACC TAGATCCTTT TAAATTAAAA ATGAAGTTTT     900
AAATCAATCT AAAGTATATA TGAGTAAACT TGGTCTGACA GTTACCAATG     950
CTTAATCAGT GAGGCACCTA TCTCAGCGAT CTGTCTATTT CGTTCATCCA    1000
TAGTTGCCTG ACTCCCCGTC GTGTAGATAA CTACGATACG GGAGGGCTTA    1050
CCATCTGGCC CCAGTGCTGC AATGATACCG CCAGACCCAC GCTCACCGGC    1100
TCCAGATTTA TCAGCAATAA ACCAGCCAGC CGGAAGGGCC GAGCGCAGAA    1150
GTGGTCCTGC AACTTTATCC GCCTCCATCC AGTCTATTAA TTGTTGCCGG    1200
GAAGCTAGAG TAAGTAGTTC GCCAGTTAAT AGTTTGCGCA ACGTTGTTGC    1250
CATTGCTACA GGCATCGTGG TGTCACGCTC GTCGTTTGGT ATGGCTTCAT    1300
```

FIGURE 7B

```
TCAGCTCCGC TTCCCAACGA TCAAGGCGAG TTACATGATC CCCCATGTTG   1350
TGCAAAAAAG CGGTTAGCTC CTTCGGTCCT CCGATCGTTG TCAGAAGTAA   1400
GTTGGCCGCA GTGTTATCAC TCATGGTTAT GGCAGCACTG CATAATTCTC   1450
TTACTGTCAT GCCATCCGTA AGATGCTTTT CTGTGACTGG TGAGTACTCA   1500
ACCAAGTCAT TCTGAGAATA GTGTATGCGG CGACCGAGTT GCTCTTGCCC   1550
GGCGTCAATA CGGGATAATA CCGCGCCACA TAGCAGAACT TTAAAAGTGC   1600
TCATCATTGG AAAACGTTCT TCGGGGCGAA AACTCTCAAG GATCTTACCG   1650
CTGTTGAGAT CCAGTTCGAT GTAACCCACT CGTGCACCCA ACTGATCTTC   1700
AGCATCTTTT ACTTTCACCA GCGTTTCTGG GTGAGCAAAA ACAGGAAGGC   1750
AAAATGCCGC AAAAAAGGGA ATAAGGGCGA CACGGAAATG TTGAATACTC   1800
ATACTCTTCC TTTTTCAATA TTATTGAAGC ATTTATCAGG GTTATTGTCT   1850
CATGAGCGGA TACATATTTG AATGTATTTA GAAAAATAAA CAAATAGGGG   1900
TTCCGCGCAC ATTTCCCCGA AAAGTGCCAC CTGACGTCTA AGAAACCATT   1950
ATTATCATGA CATTAACCTA TAAAAATAGG CGTATCACGA GGCCCTTTCG   2000
TCTCGCGCGT TTCGGTGATG ACGGTGAAAA CCTCTGACAC ATGCAGCTCC   2050
CGGAGACGGT CACAGCTTGT CTGTAAGCGG ATGCCGGGAG CAGACAAGCC   2100
CGTCAGGGCG CGTCAGCGGG TGTTGGCGGG TGTCGGGGCT GGCTTAACTA   2150
TGCGGCATCA GAGCAGATTG TACTGAGAGT GCACCATAAA ATTGTAAACG   2200
TTAATATTTT GTTAAAATTC GCGTTAAATT TTTGTTAAAT CAGCTCATTT   2250
TTTAACCAAT AGGCCGAAAT CGGCAAAATC CCTTATAAAT CAAAAGAATA   2300
GCCCGAGATA GGGTTGAGTG TTGTTCCAGT TTGGAACAAG AGTCCACTAT   2350
TAAAGAACGT GGACTCCAAC GTCAAAGGGC GAAAAACCGT CTATCAGGGC   2400
GATGGCCCAC TACGTGAACC ATCACCCAAA TCAAGTTTTT TGGGGTCGAG   2450
GTGCCGTAAA GCACTAAATC GGAACCCTAA AGGGAGCCCC CGATTTAGAG   2500
CTTGACGGGG AAAGCCGGCG AACGTGGCGA GAAAGGAAGG GAAGAAAGCG   2550
AAAGGAGCGG GCGCTAGGGC GCTGGCAAGT GTAGCGGTCA CGCTGCGCGT   2600
```

FIGURE 7C

AACCACCACA CCCGCCGCGC TTAATGCGCC GCTACAGGGC GCGTACTATG 2650

GTTGCTTTGA CGTATGCGGT GTGAAATACC GCACAGATGC GTAAGGAGAA 2700

AATACCGCAT CAGGCGCCAT TCGCCATTCA GGCTGCGCAA CTGTTGGGAA 2750

GGGCGATCGG TGCGGGCCTC TTCGCTATTA CGCCAGCTGG CGAAAGGGGG 2800

ATGTGCTGCA AGGCGATTAA GTTGGGTAAC GCCAGGGTTT TCCCAGTCAC 2850

GACGTTGTAA AACGACGGCC AGTGCCAAGC TTAAGGTGCA CGGCCCACGT 2900

GGCCACTAGT ACTTCTCGAG CTCTGTACAT GTCCGCGGTC GCGACGTACG 2950

CGTATCGATG GCGCCAGCTG CAGGCGGCCG CCATATGCAT CCTAGGCCTA 3000

TTAATATTCC GGAGTATACG TAGCCGGCTA ACGTTAACAA CCGGTACCTC 3050

TAGAACTATA GCTAGCCAAT TCCATCATCA ATAATATACC TTATTTTGGA 3100

TTGAAGCCAA TATGATAATG AGGGGGTGGA GTTTGTGACG TGGCGCGGGG 3150

CGTGGGAACG GGGCGGGTGA CGTAGGTTTT AGGGCGGAGT AACTTGTATG 3200

TGTTGGGAAT TGTAGTTTTC TTAAAATGGG AAGTTACGTA ACGTGGGAAA 3250

ACGGAAGTGA CGATTTGAGG AAGTTGTGGG TTTTTTGGCT TTCGTTTCTC 3300

GGCGTAGGTT CGCGTGCGGT TTTCTGGGTG TTTTTTGTGG ACTTTAACCG 3350

TTACGTCATT TTTTAGTCCT ATATATACTC GCTCTGCACT TGGCCCTTTT 3400

TTACACTGTG ACTGATTGAG CTGGTGCCGT GTCGAGTGGT GTTTTTTTAA 3450

TAGGTTTTCT TTTTTACTGG TAAGGCTGAC TGTTAGGCTG CCGCTGTGAA 3500

GCGCTGTATG TTGTTCTGGA GCGGGAGGGT GCTATTTTGC CTAGGCAGGA 3550

GGGTTTTTCA GGTGTTTATG TGTTTTTCTC TCCTATTAAT TTTGTTATAC 3600

CTCCTATGGG GGCTGTAATG TTGTCTCTAC GCCTGCGGGT ATGTATTCCC 3650

CCCAAGCTTG CATGCCTGCA GGTCGACTCT AGAGGATCCG AAAAAACCTC 3700

CCACACCTCC CCCTGAACCT GAAACATAAA ATGAATGCAA TTGTTGTTGT 3750

TAACTTGTTT ATTGCAGCTT ATAATGGTTA CAAATAAAGC AATAGCATCA 3800

CAAATTTCAC AAATAAAGCA TTTTTTTCAC TGCATTCTAG TTGTGGTTTG 3850

TCCAAACTCA TCAATGTATC TTATCATGTC TGGATCCCCC TAGCTTGCCA 3900

FIGURE 7D

```
AACCTACAGG TGGGGTCTTT CATTCCCCCC TTTTTCTGGA GACTAAATAA   3950
AATCTTTTAT TTTATCTATG GCTCGTACTC TATAGGCTTC AGCTGGTGAT   4000
ATTGTTGAGT CAAAACTAGA GCCTGGACCA CTGATATCCT GTCTTTAACA   4050
AATTGGACTA ATCGCGGGAT CAGCCAATTC CATGAGCAAA TGTCCCATGT   4100
CAACATTTAT GCTGCTCTCT AAAGCCTTGT ATCTTGCATC TCTTCTTCTG   4150
TCTCCTCTTT CAGAGCAGCA ATCTGGGGCT TAGACTTGCA CTTGCTTGAG   4200
TTCCGGTGGG GAAAGAGCTT CACCCTGTCG GAGGGGCTGA TGGCTTGCCG   4250
GAAGAGGCTC CTCTCGTTCA GCAGTTTCTG GATGGAATCG TACTGCCGCA   4300
CTTTGTTCTC TTCTATGACC AAAAATTGTT GGCATTCCAG CATTGCTTCT   4350
ATCCTGTGTT CACAGAGAAT TACTGTGCAA TCAGCAAATG CTTGTTTTAG   4400
AGTTCTTCTA ATTATTTGGT ATGTTACTGG ATCCAAATGA GCACTGGGTT   4450
CATCAAGCAG CAAGATCTTC GCCTTACTGA GAACAGATCT AGCCAAGCAC   4500
ATCAACTGCT TGTGGCCATG GCTTAGGACA CAGCCCCCAT CCACAAGGAC   4550
AAAGTCAAGC TTCCCAGGAA ACTGTTCTAT CACAGATCTG AGCCCAACCT   4600
CATCTGCAAC TTTCCATATT TCTTGATCAC TCCACTGTTC ATAGGGATCC   4650
AAGTTTTTTC TAAATGTTCC AGAAAAAATA AATACTTTCT GTGGTATCAC   4700
TCCAAAGGCT TTCCTCCACT GTTGCAAAGT TATTGAATCC CAAGACACAC   4750
CATCGATCTG GATTTCTCCT TCAGTGTTCA GTAGTCTCAA AAAAGCTGAT   4800
AACAAAGTAC TCTTCCCTGA TCCAGTTCTT CCCAAGAGGC CCACCCTCTG   4850
GCCAGGACTT ATTGAGAAGG AAATGTTCTC TAATATGGCA TTTCCACCTT   4900
CTGTGTATTT TGCTGTGAGA TCTTTGACAG TCATTTGGCC CCCTGAGGGC   4950
CAGATGTCAT CTTTCTTCAC GTGTGAATTC TCAATAATCA TAACTTTCGA   5000
GAGTTGGCCA TTCTTGTATG GTTTGGTTGA CTTGGTAGGT TTACCTTCTG   5050
TTGGCATGTC AATGAACTTA AAGACTCGGC TCACAGATCG CATCAAGCTA   5100
TCCACATCTA TGCTGGAGTT TACAGCCCAC TGCAATGTAC TCATGATATT   5150
CATGGCTAAA GTCAGGATAA TACCAACTCT TCCTTCTCCT TCTCCTGTTG   5200
```

FIGURE 7E

```
TTAAAATGGA AATGAAGGTA ACAGCAATGA AGAAGATGAC AAAAATCATT   5250
TCTATTCTCA TTTGGAACCA GCGCAGTGTT GACAGGTACA AGAACCAGTT   5300
GGCAGTATGT AAATTCAGAG CTTTGTGGAA CAGAGTTTCA AAGTAAGGCT   5350
GCCGTCCGAA GGCACGAAGT GTCCATAGTC CTTTTAAGCT TGTAACAAGA   5400
TGAGTGAAAA TTGGACTCCT GCCTTCAGAT TCCAGTTGTT TGAGTTGCTG   5450
TGAGGTTTGG AGGAAATATG CTCTCAACAT AATAAAAGCC ACTATCACTG   5500
GCACTGTTGC AACAAAGATG TAGGGTTGTA AAACTGCGAC AACTGCTATA   5550
GCTCCAATCA CAATTAATAA CAACTGGATG AAGTCAAATA TGGTAAGAGG   5600
CAGAAGGTCA TCCAAAATTG CTATATCTTT GGAGAATCTA TTAAGAATCC   5650
CACCTGCTTT CAACGTGTTG AGGGTTGACA TAGGTGCTTG AAGAACAGAA   5700
TGTAACATTT TGTGGTGTAA AATTTTCGAC ACTGTGATTA GAGTATGCAC   5750
CAGTGGTAGA CCTCTGAAGA ATCCCATAGC AAGCAAAGTG TCGGCTACTC   5800
CCACGTAAAT GTAAAACACA TAATACGAAC TGGTGCTGGT GATAATCACT   5850
GCATAGCTGT TATTTCTACT ATGAGTACTA TTCCCTTTGT CTTGAAGAGG   5900
AGTGTTTCCA AGGAGCCACA GCACAACCAA AGAAGCAGCC ACCTCTGCCA   5950
GAAAAATTAC TAAGCACCAA ATTAGCACAA AAATTAAGCT CTTGTGGACA   6000
GTAATATATC GAAGGTATGT GTTCCATGTA GTCACTGCTG GTATGCTCTC   6050
CATATCATCA AAAAAGCACT CCTTTAAGTC TTCTTCGTTA ATTTCTTCAC   6100
TTATTTCCAA GCCAGTTTCT TGAGATAACC TTCTTGAATA TATATCCAGT   6150
TCAGTCAAGT TTGCCTGAGG GGCCAGTGAC ACTTTTCGTG TGGATGCTGT   6200
TGTCTTTCGG TGAATGTTCT GACCTTGGTT AACTGAGTGT GTCATCAGGT   6250
TCAGGACAGA CTGCCTCCTT CGTGCCTGAA GCGTGGGGCC AGTGCTGATC   6300
ACGCTGATGC GAGGCAGTAT CGCCTCTCCC TGCTCAGAAT CTGGTACTAA   6350
GGACAGCCTT CTCTCTAAAG GCTCATCAGA ATCCTCTTCG ATGCCATTCA   6400
TTTGTAAGGG AGTCTTTTGC ACAATGGAAA ATTTTCGTAT AGAGTTGATT   6450
GGATTGAGAA TAGAATTCTT CCTTTTTTCC CCAAACTCTC CAGTCTGTTT   6500
```

FIGURE 7F

AAAAGATTGT TTTTTTGTTT CTGTCCAGGA GACAGGAGCA TCTCCTTCTA 6550

ATGAGAAACG GTGTAAGGTC TCAGTTAGGA TTGAATTTCT TCTTTCTGCA 6600

CTAAATTGGT CGAAAGAATC ACATCCCATG AGTTTTGAGC TAAAGTCTGG 6650

CTGTAGATTT TGGAGTTCTG AAAATGTCCC ATAAAAATAG CTGCTACCTT 6700

CATGCAAAAT TAATATTTTG TCAGCTTTCT TTAAATGTTC CATTTTAGAA 6750

GTGACCAAAA TCCTAGTTTT GTTAGCCATC AGTTTACAGA CACAGCTTTC 6800

AAATATTTCT TTTTCTGTTA AAACATCTAG GTATCCAAAA GGAGAGTCTA 6850

ATAAATACAA ATCAGCATCT TTGTATACTG CTCTTGCTAA AGAAATTCTT 6900

GCTCGTTGAC CTCCACTCAG TGTGATTCCA CCTTCTCCAA GAACTATATT 6950

GTCTTTCTCT GCAAACTTGG AGATGTCCTC TTCTAGTTGG CATGCTTTGA 7000

TGACGCTTCT GTATCTATAT TCATCATAGG AAACACCAAA GATGATATTT 7050

TCTTTAATGG TGCCAGGCAT AATCCAGGAA AACTGAGAAC AGAATGAAAT 7100

TCTTCCACTG TGCTTAATTT TACCCTCTGA AGGCTCCAGT TCTCCCATAA 7150

TCATCATTAG AAGTGAAGTC TTGCCTGCTC CAGTGGATCC AGCAACCGCC 7200

AACAACTGTC CTCTTTCTAT CTTGAAATTA ATATCTTTCA GGACAGGAGT 7250

ACCAAGAAGT GAGAAATTAC TGAAGAAGAG GCTGTCATCA CCATTAGAAG 7300

TTTTTCTATT GTTATTGTTT TGTTTTGCTT TCTCAAATAA TTCCCCAAAT 7350

CCCTCCTCCC AGAAGGCTGT TACATTCTCC ATCACTACTT CTGTAGTCGT 7400

TAAGTTATAT TCCAATGTCT TATATTCTTG CTTTTGTAAG AAATCCTGTA 7450

TTTTGTTTAT TGCTCCAAGA GAGTCATACC ATGTTTGTAC AGCCCAGGGA 7500

AATTGCCGAG TGACCGCCAT GCGCAGAACA ATGCAGAATG AGATGGTGGT 7550

GAATATTTTC CGGAGGATGA TTCCTTTGAT TAGTGCATAG GGAAGCACAG 7600

ATAAAAACAC CACAAAGAAC CCTGAGAAGA AGAAGGCTGA GCTATTGAAG 7650

TATCTCACAT AGGCTGCCTT CCGAGTCAGT TTCAGTTCTG TTTGTCTTAA 7700

GTTTTCAATC ATTTTTTCCA TTGCTTCTTC CCAGCAGTAT GCCTTAACAG 7750

ATTGGATGTT CTCGATCATT TCTGAGGTAA TCACAAGTCT TTCACTGATC 7800

FIGURE 7G

```
TTCCCAGCTC TCTGATCTCT GTACTTCATC ATCATTCTCC CTAGCCCAGC   7850
CTGAAAAAGG GCAAGGACTA TCAGGAAACC AAGTCCACAG AAGGCAGACG   7900
CCTGTAACAA CTCCCAGATT AGCCCCATGA GGAGTGCCAC TTGCAAAGGA   7950
GCGATCCACA CGAAATGTGC CAATGCAAGT CCTTCATCAA ATTTGTTCAG   8000
GTTGTTGGAA AGGAGACTAA CAAGTTGTCC AATACTTATT TTATCTAGAA   8050
CACGGCTTGA CAGCTTTAAA GTCTTCTTAT AAATCAAACT AAACATAGCT   8100
ATTCTCATCT GCATTCCAAT GTGATGAAGG CCAAAAATGG CTGGGTGTAG   8150
GAGCAGTGTC CTCACAATAA AGAGAAGGCA TAAGCCTATG CCTAGATAAA   8200
TCGCGATAGA GCGTTCCTCC TTGTTATCCG GGTCATAGGA AGCTATGATT   8250
CTTCCCAGTA AGAGAGGCTG TACTGCTTTG GTGACTTCCC CTAAATATAA   8300
AAAGATTCCA TAGAACATAA ATCTCCAGAA AAAACATCGC CGAAGGGCAT   8350
TAATGAGTTT AGGATTTTTC TTTGAAGCCA GCTCTCTATC CCATTCTCTT   8400
TCCAATTTTT CAGATAGATT GTCAGCAGAA TCAACAGAAG GGATTTGGTA   8450
TATGTCTGAC AATTCCAGGC GCTGTCTGTA TCCTTTCCTC AAAATTGGTC   8500
TGGTCCAGCT GAAAAAAAGT TTGGAGACAA CGCTGGCCTT TTCCAGAGGC   8550
GACCTCTGCA TGGTCTCTCG GGCGCTGGGG TCCCTGCTAG GGCCGTCTGG   8600
GCTCAAGCTC CTAATGCCAA AGGAATTCCT GCAGCCCGGG GGATCCACTA   8650
GTTCTAGAGC GGCCGCCACC GCGGTGGCTG ATCCCGCTCC CGCCCGCCGC   8700
GCGCTTCGCT TTTTATAGGG CCGCCGCCGC CGCCGCCTCG CCATAAAAGG   8750
AAACTTTCGG AGCGCGCCGC TCTGATTGGC TGCCGCCGCA CCTCTCCGCC   8800
TCGCCCCGCC CCGCCCCTCG CCCCGCCCCG CCCCGCCTGG CGCGCGCCCC   8850
CCCCCCCCCC CCGCCCCCAT CGCTGCACAA AATAATTAAA AAATAAATAA   8900
ATACAAAATT GGGGGTGGGG AGGGGGGGGA GATGGGGAGA GTGAAGCAGA   8950
ACGTGGCCTC GAGTAGATGT ACTGCCAAGT AGGAAAGTCC CATAAGGTCA   9000
TGTACTGGGC ATAATGCCAG GCGGGCCATT TACCGTCATT GACGTCAATA   9050
GGGGGCGTAC TTGGCATATG ATACACTTGA TGTACTGCCA AGTGGGCAGT   9100
```

FIGURE 7H

TTACCGTAAA TACTCCACCC ATTGACGTCA ATGGAAAGTC CCTATTGGCG 9150

TTACTATGGG AACATACGTC ATTATTGACG TCAATGGGCG GGGGTCGTTG 9200

GGCGGTCAGC CAGGCGGGCC ATTTACCGTA AGTTATGTAA CGACCTGCAG 9250

GCTGATCTCC CTAGACAAAT ATTACGCGCT ATGAGTAACA CAAAATTATT 9300

CAGATTTCAC TTCCTCTTAT TCAGTTTTCC CGCGAAAATG GCCAAATCTT 9350

ACTCGGTTAC GCCCAAATTT ACTACAACAT CCGCCTAAAA CCGCGCGAAA 9400

ATTGTCACTT CCTGTGTACA CCGGCGCACA CCAAAAACGT CACTTTTGCC 9450

ACATCCGTCG CTTACATGTG TTCCGCCACA CTTGCAACAT CACACTTCCG 9500

CCACACTACT ACGTCACCCG CCCCGTTCCC ACGCCCCGCG CCACGTCACA 9550

AACTCCACCC CCTCATTATC ATATTGGCTT CAATCCAAAA TAAGGTATAT 9600

TATTGATGAT GCTAGCATGC GCAAATTTAA AGCGCTGATA TCGATCGCGC 9650

GCAGATCTGT CATGATGATC ATTGCAATTG GATCCATATA TAGGGCCCGG 9700

GTTATAATTA CCTCAGGTCG ACGTCCCATG GCCATTCGAA TTCGTAATCA 9750

TGGTCATAGC TGTTTCCTGT GTGAAATTGT TATCCGCTCA CAATTCCACA 9800

CAACATACGA GCCGGAAGCA TAAAGTGTAA AGCCTGGGGT GCCTAATGAG 9850

TGAGCTAACT CACATTAATT GCGTTGCGCT CACTGCCCGC TTTCCAGTCG 9900

GGAAACCTGT CGTGCCAGCT GCATTAATGA ATCGGCCAAC GCGCGGGGAG 9950

AGGCGGTTTG CGTATTGGGC GC 9972

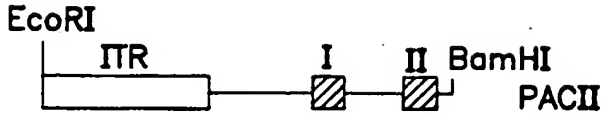


FIG. 8A

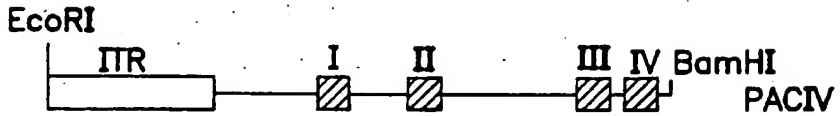


FIG. 8B

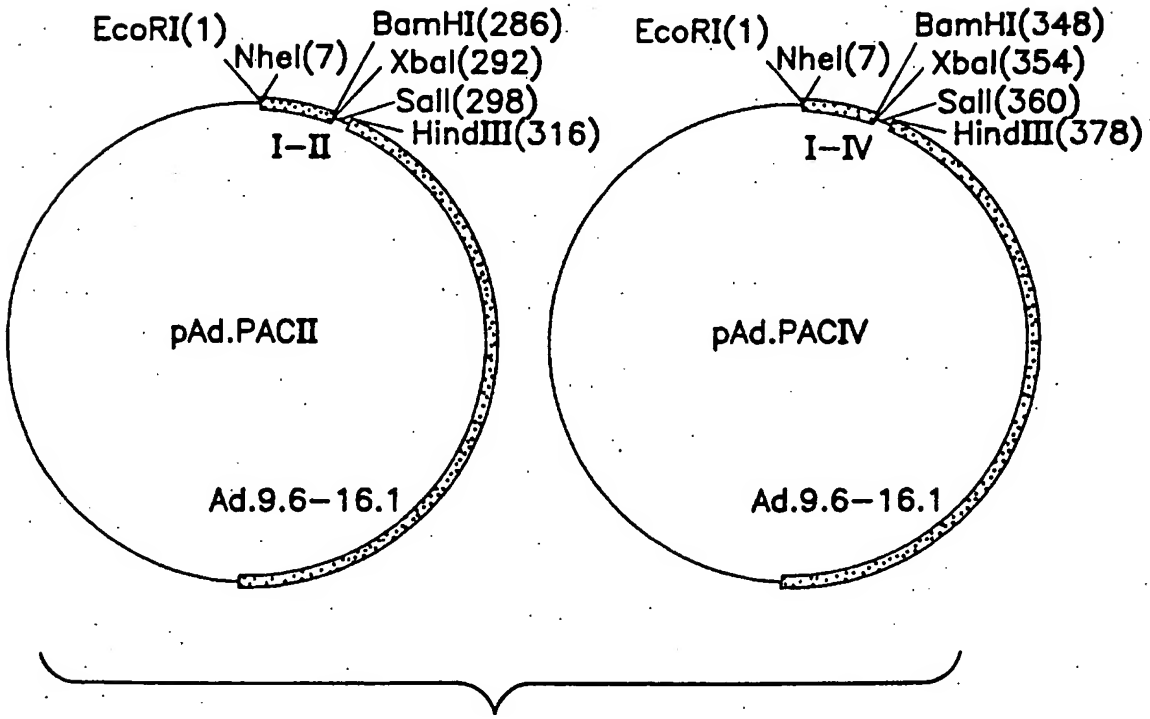
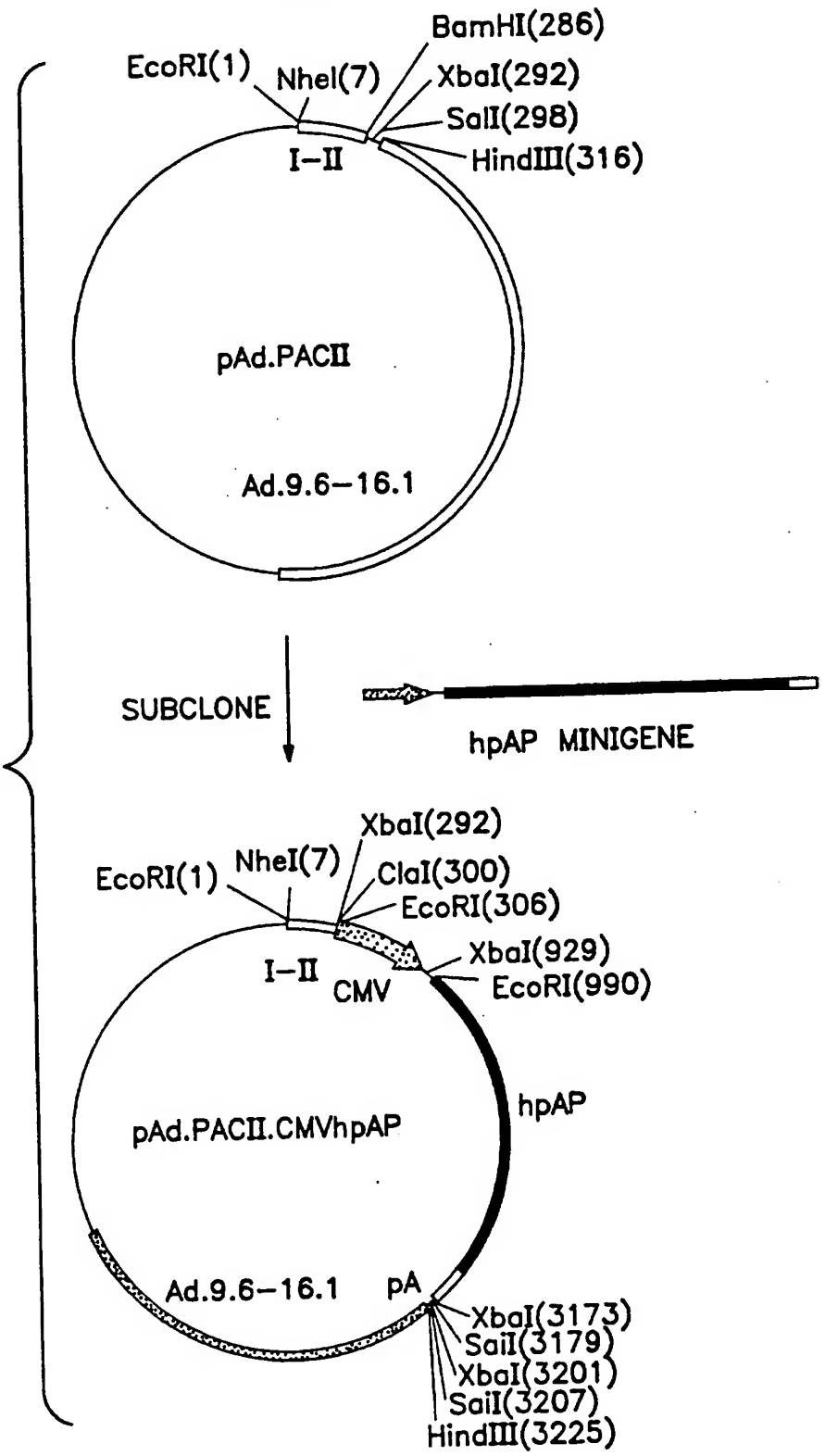


FIG. 8C

FIG. 9A


BamHI(286)
EcoRI(1)
NheI(7)
XbaI(292)
SalI(298)
HindIII(316)
I–II
pAd.PACII
Ad.9.6–16.1
SUBCLONE
hpAP MINIGENE
XbaI(292)
EcoRI(1)
NheI(7)
ClaI(300)
EcoRI(306)
XbaI(929)
I–II
CMV
EcoRI(990)
hpAP
pAd.PACII.CMVhpAP
Ad.9.6–16.1
pA
XbaI(3173)
SalI(3179)
XbaI(3201)
SalI(3207)
HindIII(3225)

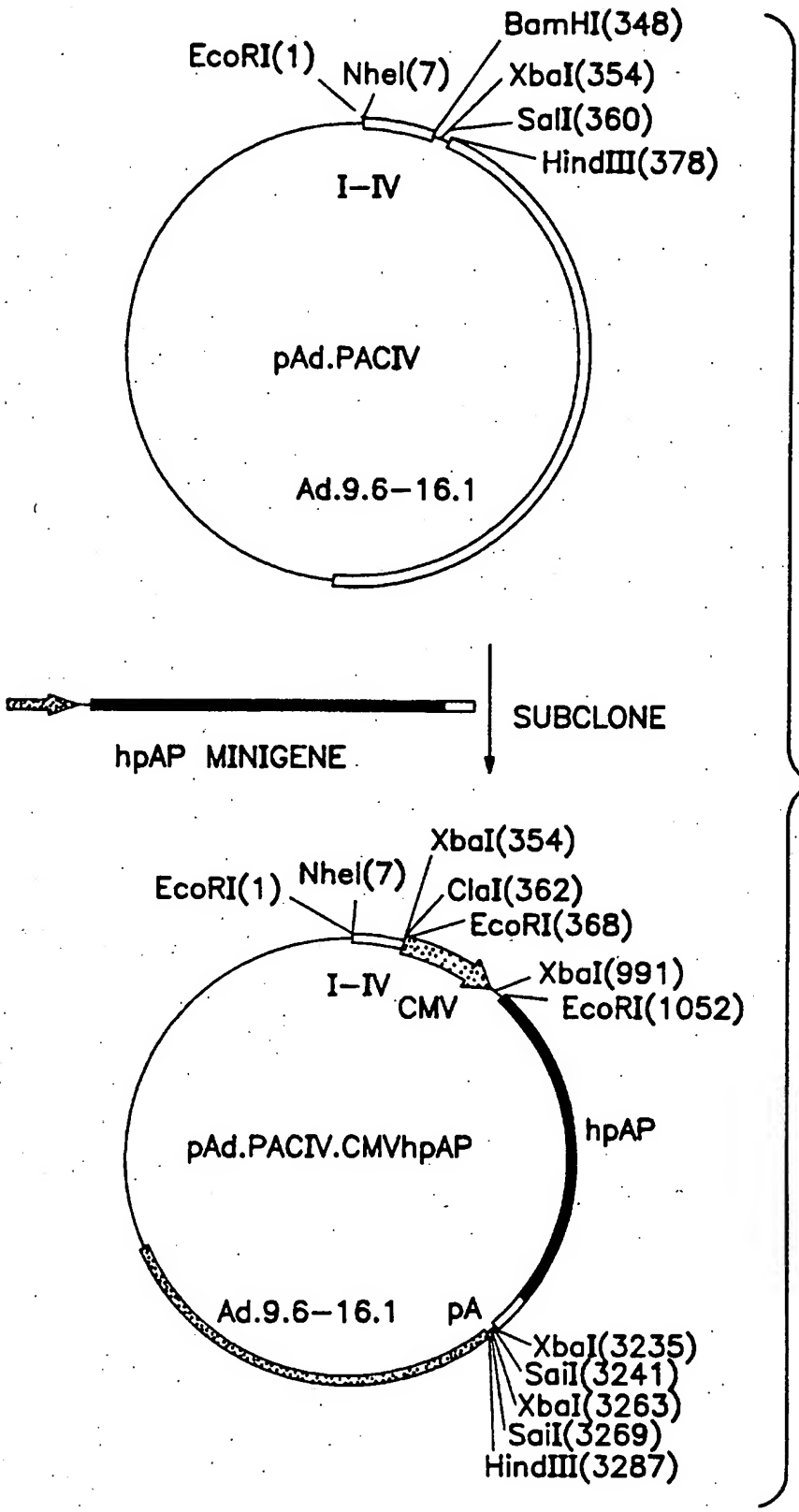
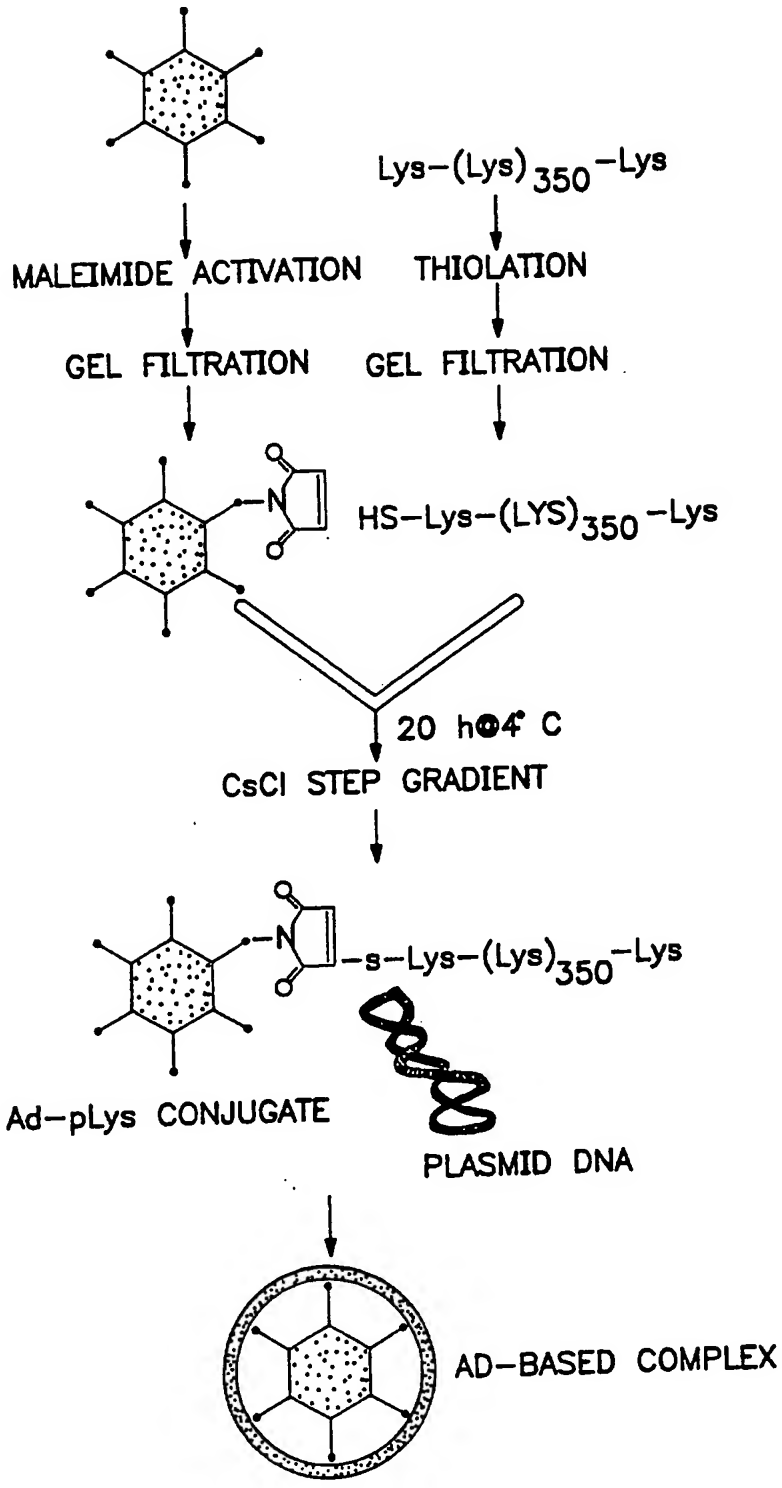

BamHI(348)
EcoRI(1)
NheI(7)
XbaI(354)
SalI(360)
HindIII(378)
I–IV
pAd.PACIV
Ad.9.6–16.1
SUBCLONE
hpAP MINIGENE
XbaI(354)
EcoRI(1)
NheI(7)
ClaI(362)
EcoRI(368)
I–IV
CMV
XbaI(991)
EcoRI(1052)
hpAP
pAd.PACIV.CMVhpAP
Ad.9.6–16.1
pA
XbaI(3235)
SalI(3241)
XbaI(3263)
SalI(3269)
HindIII(3287)

FIG. 9B

FIG. 10


Lys–(Lys)350–Lys
MALEIMIDE ACTIVATION
THIOLATION
GEL FILTRATION
GEL FILTRATION
HS–Lys–(LYS)350–Lys
20 h@4° C
CsCl STEP GRADIENT
–s–Lys–(Lys)350–Lys
Ad–pLys CONJUGATE
PLASMID DNA
AD–BASED COMPLEX

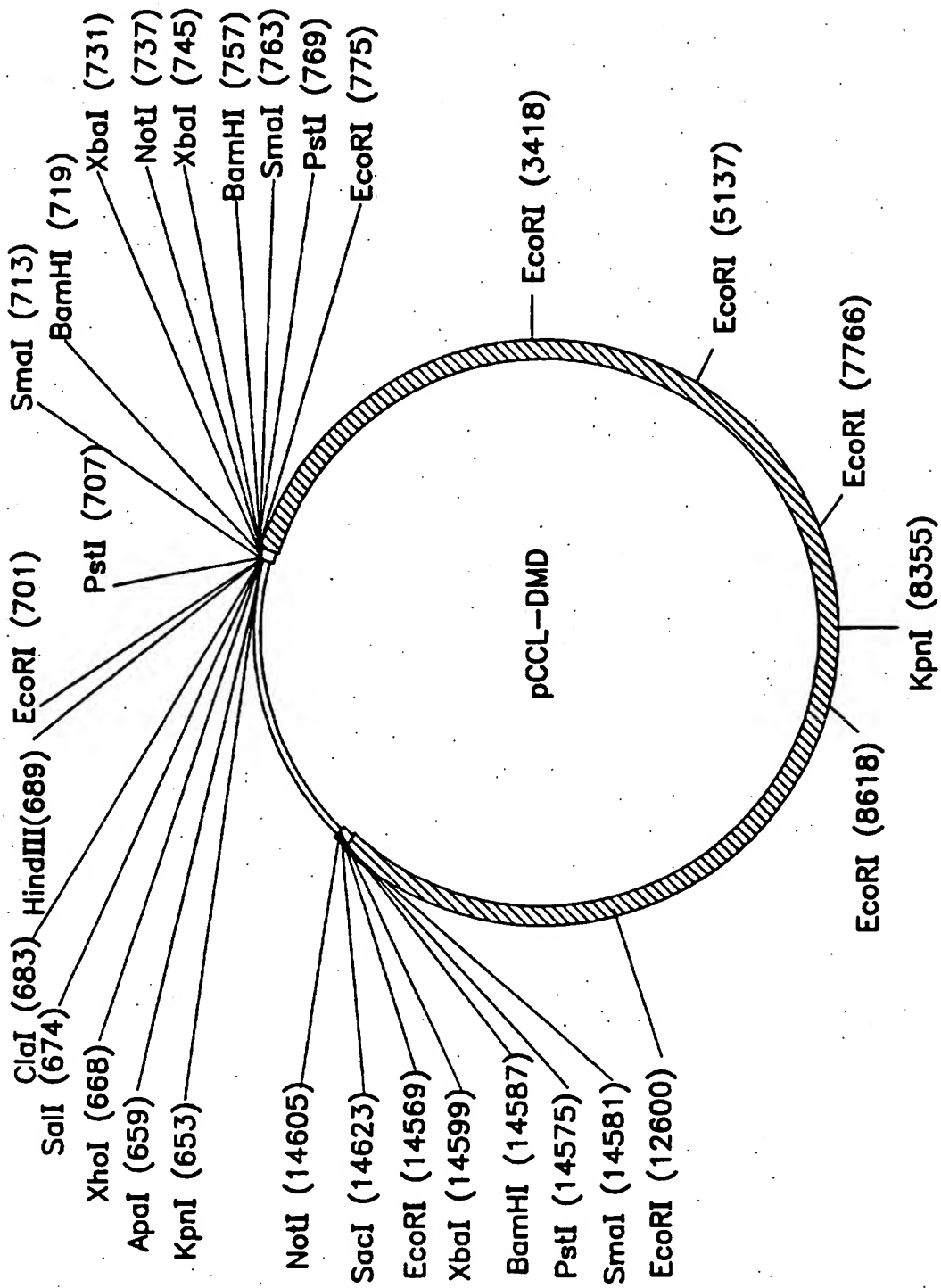

pCCL-DMD
EcoRI (3418)
EcoRI (5137)
EcoRI (7766)
KpnI (8355)
EcoRI (8618)
EcoRI (12600)
SmaI (14581)
PstI (14575)
BamHI (14587)
XbaI (14599)
EcoRI (14569)
SacI (14623)
NotI (14605)
KpnI (653)
ApaI (659)
XhoI (668)
SalI (674)
ClaI (683)
HindIII(689)
EcoRI (701)
PstI (707)
SmaI (713)
BamHI (719)
XbaI (731)
NotI (737)
XbaI (745)
BamHI (757)
SmaI (763)
PstI (769)
EcoRI (775)

FIG. 11

FIGURE 12A

```
CCAATTCCAT CATCAATAAT ATACCTTATT TTGGATTGAA GCCAATATGA      50
TAATGAGGGG GTGGAGTTTG TGACGTGGCG CGGGGCGTGG GAACGGGGCG     100
GGTGACGTAG GTTTTAGGGC GGAGTAACTT GTATGTGTTG GGAATTGTAG     150
TTTTCTTAAA ATGGGAAGTT ACGTAACGTG GGAAAACGGA AGTGACGATT     200
TGAGGAAGTT GTGGGTTTTT TGGCTTTCGT TTCTGGGCGT AGGTTCGCGT     250
GCGGTTTTCT GGGTGTTTTT TGTGGACTTT AACCGTTACG TCATTTTTTA     300
GTCCTATATA TACTCGCTCT GCACTTGGCC CTTTTTTACA CTGTGACTGA     350
TTGAGCTGGT GCCGTGTCGA GTGGTGTTTT TTTAATAGGT TTTCTTTTTT     400
ACTGGTAAGG CTGACTGTTA GGCTGCCGCT GTGAAGCGCT GTATGTTGTT     450
CTGGAGCGGG AGGGTGCTAT TTTGCCTAGG CAGGAGGGTT TTTCAGGTGT     500
TTATGTGTTT TTCTCTCCTA TTAATTTTGT TATACCTCCT ATGGGGGCTG     550
TAATGTTGTC TCTACGCCTG CGGGTATGTA TTCCCCCCAA GCTTGCATGC     600
CTGCAGGTCG ACTCTAGAGG ATCCGAAAAA ACCTCCCACA CCTCCCCCTG     650
AACCTGAAAC ATAAAATGAA TGCAATTGTT GTTGTTAACT TGTTTATTGC     700
AGCTTATAAT GGTTACAAAT AAAGCAATAG CATCACAAAT TTCACAAATA     750
AAGCATTTTT TTCACTGCAT TCTAGTTGTG GTTTGTCCAA ACTCATCAAT     800
GTATCTTATC ATGTCTGGAT CCCCGCGGCC GCTCTAGAAC TAGTGGATCC     850
CCCGGGCTGC AGGAATTCCG TAACATAACT GCGTGCTTTA TTGAGATACA     900
CAGTAAAGCA GTAATATAAT ACAATAGTAA GGCATATATT TGGTGAAATC     950
TGATATGTTG TGAAAATGCA GTAAAACTGA AGTTTAAAAA AATAATTAGT    1000
AAATGTTACA GTGTTGGTGT TAAAACACAA TCTATTATGA TACTCAAGTA    1050
AGAGTCCAGT ACCTGGAGAC AATGATGATA CATGCCATGT GATGATTATG    1100
CTTCAGTTAC ACTGATTATG ATTTACACTT TAATACTTGA TGGTTATAAA    1150
GAACATGAAA TGATGTCCAA ATTATGCTTA AAATCAGCAA TAAAGCTCTC    1200
AGTTTTTATT CAAATATTTT GATAGATTCA CTCCAGAACT AATATCTAAA    1250
```

FIGURE 12B

```
AGATAAAACG AAAAGATTAA AACAAAACTA TGCACTCTAT CTACCTTGGA    1300
TTTTAGAATG AAACTTAAAA CTTCTTAGTA GGAAAGGAAC CCCTTGTTTT    1350
AAATCTTGGT GAAAACAAAT CCTTGGATAA AGAAAATGCC CAGTGCCACA    1400
TAAAGGAGAG AGAGAGAGAA AAGCAAGACC AGAACCAAAT TTCAATTTGT    1450
TATCTTAGAG CTTTGGGTTT TCTTTTGGAA ATTATAAATG AAAAAAGGAA    1500
ACTGGTGTCC ACACAACAGA CAAGTGGTGA AGTTGTGAAA TTAGGTGTGC    1550
ACAATTACTA GAAACACCCC AAAACCAAAG TGAGGTAGAA ATAGCATGAG    1600
AAGCTGTGTT TGATGTTAAT TACAATTAAT AATGGACAAA ACCCACTCGC    1650
TAGAAGTTAA TTACACTTGA CGTTAGAGGT AACAGATTTG CAAAATGATA    1700
GGACAGTGAT TTCTATTGAG AGAATGCTCT TTAAATGCTA AGAAGAAGAA    1750
ACTGGCATGA GAGGAGTAAA GCTCTTCCTA GCAGTCCTTA GCTTTCTGTT    1800
GCACTTTTTC TCCTGGTTCA ATGACTTGCA TTTGTTTAGA CATTTCAGCC    1850
CGTCAACTAG ACCAGAGAGT TTGGAGACGC TTTTGCTCTC AAAACTTTCC    1900
AACCACTGTG CCTTCTCACC CACAATCCTG TGTGGAGTTA CTTGCAGGGA    1950
AACCAATGCA AAGGAGACAA ATGCAGTTCA TGGGCTTCTG GACTGATATT    2000
CACCAGGGTC ACAATGTGAT TGGGTTACTT TCTTAACAGT AATCCTAAGT    2050
CTTGCAGCAT TAAAAAAAAA AATCATCACA ATGAAGAAAA AAAAACCCAA    2100
AAAATCTAAA ATCTAAAATT CATCATCATC ATCAACAACA ACAACAACAA    2150
CAACAACAAA ACCACCCACT TCAGGTTGAG TTTATGAAGA GGGCAGAACA    2200
ATTTAGTTGT AATTATAGAG ATGTTTATAT GTATAGTTGT AAATATTCAT    2250
CCATTCTTTT ACAGAGTTGT TGCTCCCCTC ATATAAATTG ACTGAGGAGC    2300
CGCAACCTTT AGCTCCTACC ATCTTCCTCC TACTGTCTGG GAGTTAAAAA    2350
TGTCATCTGA TGTTCTATTG CAGAAACATC ATTAAATATA ACCCAACAGT    2400
AGGAAGTTGA ATATATCAGC CAACAAATTA CTATGATAGT AAGTCCTGTG    2450
TATTCATTCG CATGTTCCTT GAAAAAAATG AATCCTCTAG CTCTCAGTGG    2500
```

FIGURE 12C

AAAGTTTAAA ACTAGAAACA TCTGGAGCCC TAGACAATAT TTTAGTGTGG 2550

CGGTAGTCTC CTGGCTTTGG GCTCCAGGGA AAATTCACTC TTGCCCAAGC 2600

AGATAAGCCC AGATGACTAG AAGCAATTTC CATTAGGAAG TGGCAAGAAC 2650

ATTTGAAGAA GTAACTTCAT ATCTATTTAT CTATATACCT ATAGTATTTA 2700

TATACTTGTA GACATATAGA TGTATAAAAT GAAAGCCCAT AGCCAGCCCC 2750

ACTCAGTCAA CAATTCTCAA AAGAGCAATA TGAAGCAGTC ATTTGGTGGG 2800

GTTCGTATGC AAGAAAATAA AAAAACGTCA TGAATTCCAT ATGAATACCA 2850

CGCTAAAGTA ATGCAAAACA ATGTGCTGCC TCAGTGTGTG TGTGTGTGTG 2900

TGTGTGTGTG GTGGGTTCGT GCATGTATGT GTGCGTGTGT GTGTGTGTGT 2950

GTGTGTGTGT GTGTGTGTGC GTGTGTGTTT GTTTAGGGGT TTTTATAAAC 3000

AACTTTTTTT ATAAAGCACA CTTTAGTTTA CAATCTCTCT TTATAACTGT 3050

TATAAATTTT TAAACAACCC AAAATGCGTT CCATATAAAG AAATGGCAAG 3100

TTATTTAGCT ATCAAGATTT TACATGTTTT CTTTTAACTT TTTTGTACAA 3150

TTGCATAGAC GTGTAAAACC TGCCATTGTT AACAAAACAA TAACAGACTT 3200

AGAAACTACT GAAATCTACA GTATAGTACC ACTACCCTTC ACAAAAATAT 3250

AGATTTTATT TCTTGTAAAC TCTTACTGTC TAATCCTCTT TGTTGTACGA 3300

ATATTATAAA AACCATGCGG GAATCAGGAG TTGTAAAACA TTTATTCTGC 3350

TCCTTCTTCA TCTGTCATGA CTGAAACTAA GGACTCCATC GCTCTGCCCA 3400

AATCATCTGC CATGTGGAAA AGGCTTCCTA CATTGTGTCC TCTCTCATTG 3450

GCTTTCCGGG GGCATTTCTT CCTCTTGAAC TAGGGAAGGA GTTGTTGAGT 3500

TGCTCCATCA CTTCTTCTAA CCCTGTGCTT GTGTCCTGGG GAGGACTCAG 3550

AAGATCTTCC TCACCCATAG ATTCTGAAGT TTGACTGCCA ACCACTCGGA 3600

GCAGCATAGG CTGACTGCTA TCTGACCTCT GCAGAGAGGT GGAAGGAGAG 3650

GACACCGTGG TGCCATTCAC CTTAGCTTCA GCCTGGGGCT GCTCCAGGAG 3700

CTGTCTCAGT CTATGTAACT GAGACTCCAG CTGTTTATTG TGGTCTTCCA 3750

FIGURE 12D

```
GGATTTGCAT CCTGGCTTCC AGGCGTCCTT TGTGTTGGCG CAGTAGCTTA  3800
GCCTCAGCAA TGAGCTCAGC ATCCCTGGGA CTCTGAGGAG AGGTGGGCAT  3850
CATCTCAGGA GGAGATGGCA GTGGAGACAG GCCTTTATGC TCATGCTGCT  3900
GCTTCAGGCG ATCATATTCT GCTTGCAGAT TCCTGTTTTC TTCCTCAAGA  3950
TCTGCTAGGA TTCTCTCTAG CTCCCCTCTT TCCTCACTCT CTAAGGAAAT  4000
CAAGATCTGG GCAGGACTAC GAGGCTGGCT CAGGGGGGAG TCCTGGTTCA  4050
AACTTTGGCA GTAATGCTGG ATTAACAAAT GTTCATCATC TATGCTCTCA  4100
TTAGGAGAGA TGCTATCATT TAGATAAGAT CCATTGCTGT TTTCCATTTC  4150
TGCTAGCCTG CTAGCATAAT GTTCAATGCG TGAATGAGTA TCATCGTGTG  4200
AAAGCTGGGG GGACGAGGCA GGCGCAGAAT CTACTGGCCA GAAGTTGATC  4250
AGAGTAACGG GAGTTTCCAT GTTGTCCCCC TCTAACACAG TCTGCACTGG  4300
CAGGTAGCCC ATTCGGGGAT GCTTCGCAAA ATACCTTTTG GTTCGAAATT  4350
TGTTTTTTAG TACCTTGGCG AAGTCGCGAA CATCTTCTCC GGATGTAGTC  4400
GGAGTGCAAT ACTCTACCAT GGGGTAGTGC ATTTTATGGC CCTTTGCAAC  4450
TCGGCCAGAA AAAAAGCAAC TTTGGCAGAT GTCATAATTA AAATGCTTTA  4500
GGCTTCTGTA CCTGAATCCA ATGATTGGAC ACTCCTTACA GATGTTACAC  4550
TTGGCTTGAT GCTTGGCAGT TTCAGCAGCA GCCACTCTGT GCAAGACGGG  4600
CAGCCACACC ATAGACTGGG GTTCCAGGCG CATCCAGTCA AGGAAGAGAG  4650
CAGCTTCAAT CTCAGGTTTA TTATTGGCAA ATTGGAAGCA GCTCCTGACA  4700
CTCGGCTCAA TGTTACTGCC CCCAAAGGAA GCAACTTCAC CCAACTGTCT  4750
TGGGATTTGA ATAGAATCAT GCAGAAGAAG ACCCAGCCTA CGCTGGTCAC  4800
AAAAGCCAGT TGAACTTGCC ACTTGCTTGA AAAGGTATCT GTACTTGTCT  4850
TCCAAGTGTG CTTTACACAG AGAAATGATG CCAGTTTTAA AAGACAGGAC  4900
ACGGATCCTC CCTGTTCGTC CCGTATCATA AACATTGAGA AGCCAGTTGA  4950
GACACATATC CACACAGAGA GGGACATTGA CCAGATTGTT GTGCTCTTGC  5000
TCCAGACGAT CATAAATTGT AGTCAAACAG TTAATTATCT GCAGGATATC  5050
```

FIGURE 12E

CATGGGCTGG TCATTTTGCT TGAGGTTGTG CTGGTCCAGG GCATCACATG 5100

CAGCTGACAG GCTCAAGAGA TCCAAGCAAA GGGCCTTCTG GAGCCTTCTG 5150

AGCTTCATGG CAGTCCTATA CGCGGAGAAC CTGACATTAT TCAGGTCAGC 5200

TAAAGACTGG TAGAGCTCTG TCATTTTGGG GTGGTCCCAA CAAGTGGTTT 5250

GGGTCTCGTG GTTGATATAG TAGGGCACTT TGTTTGGTGA GATGGCTCTC 5300

TCCCAGGGAC CCTGAACTGA AGTGGAAAGG AAGTGCTGGG ATGCAGGACC 5350

AAAGTCCCTG TGGGCTTCAT GCAGCTGTCT GACACGGTCC TCCACAGCCA 5400

CCTGTAGAAG CCTCCATCTG GTATTCAGAT CTTCCAAAGT GCTGAGGTTA 5450

TAAGGTGAGA GCTGAATGCC CAGTGTGGTC AGCTGATGTG CAAGGTCATT 5500

GACACGATTG ACATTCTCTT TAAGAGGTGC AATTTCTCCC CGAAGTGCCT 5550

TGACTTTTTC AAGGTGATCT TGCAGAGAGT CAATGAGGAG ATCCCCCACT 5600

GGCTGCCAGG ATCCCTTGAT CACCTCAGCT TGGCGCAACT TGAGGTCCAG 5650

TTCATCGGCA GCTTCCTGAA GTTCCTGGAG TCTTTCAAGA GCTTCATCTA 5700

TTTTTCTCTG CCAATCAGCT GAGCGCAGGT TCAATTTGTC CCATTCAGCG 5750

TTGACCTCTT CAGCCTGCTT TCGTAGGAGC CGAGTGACAT TCTGAGCTCT 5800

TTCTTCAGGA GGCAGTTCTC TGGGCTCCTG GTAGAGTTTC TCTAGTCCTT 5850

CCAAAGGCTG CTCTGTCAGA AATATTCTCA CAGTCTCCAG AGTACTCATG 5900

ATTACAGGTT CTTTAGTTTT CAATTCCCTC TTGAAGGCCC TATGTATATC 5950

ATTCTGCTTC TGAACTGCTG GGAAATCACC ACCGATGGGT GCCTGACGGC 6000

TCAGTTCATC ATCTTTCAGC TGTAGCCAAA CAAGAAGTTC CTGAAGAGAA 6050

AGATGCAAAC GCTTCCACTG GTCAGAACTT GCTTCCAAAT GGGACCTAAT 6100

GTTGAGAGAC TTTTTCTGAA GTTCACTCCA CTTGAAATTC ATGTTATCCA 6150

AACGTCTTTG TAACAGGGGT GCTTCATCCG AACCTTCCAG GGATCTCAGG 6200

ATTTTTTGGC CATTTTCATC AAGATTGTGA TAGATATCTG TGTGAGTTTC 6250

AATTTCTCCT TGGAGATCTT GCCATGGTTT CATCAGCTCT CTGACTCCCC 6300

TGGAGTCTTC TAGGAGCTTC TCCTTACGGG AAGCGTCCTG TAGGACATTG 6350

FIGURE 12F

GCAGTTGTTT CTGCTTCCGT AATCCAGGAA AGAAACTTCT CCAGGTCCAG 6400

AGGGAACTGC TGCAGTAATC TATGAGTTTC TTCCAAAGCA GCCTCTTGCT 6450

CACTTACTCT TTTATGAATG TTTCCCCAAG AAGTATTGAT ATTCTCTGTT 6500

ATCATGTGTA CTTTTCTGGT ATCATCAGCA GAATAGTCCC GAAGAAGTTT 6550

CAGTGCCAAA TCATTTGCCA CGTCTACACT TATCTGCCGT TGACGGAGGT 6600

CTTTGGCCAA CTGCTTGGTT TCTGTGATCT TCTTTTGGAT TGCATCTACT 6650

GTGTGAGGAC CTTCTTTCCA TGAGTCAAGC TTGCCTCTGA CCTGTCCTAT 6700

GACCTGTTCG GCTTCTTCCT TAGCTTCCAG CCATTGTGTT GAATCCTTTA 6750

ACATTTCATT CAACTGTTGT CTCCTGTTCT GCAGCTGTTC TTGAACCTCA 6800

TCCCACTGAA TCTGAATTCT TTCAATTCGA TCAGTAATGA TTGTTCTAGC 6850

TTCTTGATTG CTGGTTTTGT TTTTCAAATT CTGGGCAGCA GTAATGAGTT 6900

CTTCCAATTG GGGGCGTCTC TGTTCCAAAT CTTGCAGTGT TGCCTTCTGT 6950

TTGATGATCA TTTCATTGAT GTCTTCCAGA TCACCCACCA TCACTCTCTG 7000

TGATTTTATA ACTCGATCAA GCAGAGACAG CCAGTCTGTA AGTTCTGTCC 7050

AAGCTCGGTT GAAGTCTGCC AGTGCAGGTA CCTCCAACAG CAAAGAAGAT 7100

GGCATTTCTA GTTTGGAGAT GACAGTTTCC TTAGTAACCA CAGATTGTGT 7150

CACTAGAGTA ACAGTCTGAC TGGCAGAGGC TCCAGTAGTG CTCAGTCCAG 7200

GGGCACGGTC AGGCTGCTTT GTCCTCAGCT CCCGAAGTAA ATGGTTTACA 7250

GCCTCCCACT CAGACCTCAG ATCTTCTAAC TTCCTCTTCA CTGGCTGAGT 7300

GCTTGGTTTT TCCTTATACA AATGCTGCCC TTTCGACAAA AGCCTTTCCA 7350

CATCCGCTTG TTTACCGTGA ACTGTTACTT CAATCTCCTT TATGTCAAAC 7400

GGTCCTGCCT GACTTGGTTG GTTATAAATT TCCAACTGGT TTCTAATAGG 7450

AGAGACCCAC AGAAGCAGGT GATCCAGCTG CTCTTCAAGC TGCCTAAAAT 7500

CTTTTAAGTG AACCTCAAGC TCTCCTTGTT TCTCAGGTAA AGCTCTGGAG 7550

ACCTTTATCC ACTGGAGATT TGTCTGTTTG AGCTTCTTTT CAAGTTTATC 7600

FIGURE 12G

TTGCTCTTCT GGCCTTATGG GAGCACTTAC AAGTACTGCT CCTCCTGTTT 7650

CATTTAATTG TTTTAGAATT CCCTGGCGCA GGGGCAACTC TTCTGCCAGT 7700

AACTTGACTT GTTCAAGTTG TTCTTTTAGC TGCTGCTCAT CTCCAAGTGG 7750

AGTAATAGCA ATGTTATCTG CTTCTTCCAG CCACAAAACA AATTCATTTA 7800

AATCTCTTTG AAATTCTGAC AAGACATTCT TTTGTTCTTC AATCCTCTTT 7850

CTCCTTTCTG CCAGCTCTTT GCAGATGTCG TGCCACCGCA GACTCAAGCT 7900

TCCTAATTTT TCTTGTAGAA TATTGACATC TGTTTTTGAA GACTGTTGAA 7950

TTATTTCTTC CCCAGTTGCA TTCAGTGTTC TGACAACAGC TTGACGCTGC 8000

CCAATGCCAT CCTGGAGTTC CTTAAGATAC CATTTGTATT TAGCATGTTC 8050

CCAGTTTTCA GGATTTTGTG TCTTTTTGAA AAACTGTTCA ACTTCATTCA 8100

GCCATTGATT AAATACCTTC ATATCATAAT GAAAGTGTCG CCATTTTTCA 8150

ACTGATCTGT CGAATCGCCC TTGTCGTTCC TTGTACATTC TATGAAGTTT 8200

TTCCCCCTGG AAATCCATCT GTGCCACGGC TTCCTGTACT TTCACCTTTT 8250

CCATGGAGGT GGCACTTTGC AAGGCTGCTG TCTTCTTCTT GTGAATAATA 8300

TCAATCCGAC CTGAGATTTG TTGCAAATTG TCTTTTATAT TCTTAAGAGA 8350

CTCCTCTTGC TTAAAAAGAT CTTCAAAATC TTTAGCACAG AGTTCAGGAG 8400

TATTTAGAAG ATGATCAACT TCTGAAAGAG CTTGTAAGAT ATGACTGATC 8450

TCGGTCAAAT AAGTAGAAGG CACATAAGAA ACATCCAAAG GCATATCTTC 8500

AGTCGTCACT ACCATAGTTT CTTCATGGAG AGTGTGAATT TGTGCAAAGT 8550

TGAGTCTTCG AAACTGAGCA AAATTGCTCT CAATTTGCCG CCAGCGCTTG 8600

CTGAGCTGGA TCTGAGTTGG CTCCACTGCC ATTGCGGCCC CATTCTCAGA 8650

CAAGCCCTCA GCTTGCCTGC GCACTGCATT CAGCTCCTCT TTCTTCTTCT 8700

GCAATTCACG ATCAATTTCC TTTAATTTTC TTTCATCTCT GGGTTCAGGT 8750

AGGCTGGCTA ATTTTTTTTC AATTTCATCC AAGCATTTCA GGAGATCATC 8800

AGCCTGCCTC TTGTACTGAT ACCACTGGTG AGAAATTTCT AGGGCCTTTT 8850

FIGURE 12H

```
TTCTTCTTTG AGACCTCAAA TCCTTGAGAG CATTATGTTT TGTCTGTAAC    8900
AGCTGCTGTT TTATCTTTAT TTCCTCTCGC TTTCTCTCAT CTGTGATTCT    8950
TTGTTGTAAG TTGTCTCCTC TTTGCAACAA TTCATTTACA GTACCCTCAT    9000
TGTCTTCACT CATATCTTTA TTGAAGTCTT CCTCTTTCAG ATTCACCCCC    9050
TGCTGAATTT CAGCCTCCAG TGGTTCAAGC AATTTTTGTA TATCTGAGTT    9100
AAACTGCTCC AATTCCTTCA AAGGAATGGA GGCCTTTCCA GTCTTAATTC    9150
TGTGAGAAAT AGCTGCAAAT CGACGGTTGA GCTCAGAGAT TTGGGGCTCT    9200
ACTACTTTCC TGCAGTGGTC ACCGCGGTTT GCCATCAATT TTGCTGCTTG    9250
GTCACGTGTG GAGTCCACCT TTGGGCGCAT GTCATTCATT TCAGCCTTTA    9300
AACGCTTAAG AATGTCTTCC TTTTGTTGTG GTTTCTTCTT TTCAGACTCA    9350
TCTAAAAGTT CATCTGCATG AATGATCCAC TTTGTGATTT GTTCTATGTT    9400
CTGATCAAAG GTTTCCATGT GTTTCTGGTA TTCCAACAAA AGATTTAGCC    9450
ATTCTTCTAC TCTGGAGGTG ACAGCTATCC AGTTACTGTT CAGAAGACTC    9500
AGTTTATCTT CTACCAAGGT TTCTTTCTTG CCCAACACCA TTTTCAAAGA    9550
CTCTCCTAAT TCTGTAACAC TCTTCAAGTG AGCCTTCTGT TTCTCAATCT    9600
CTTTTTGAGT AGCCTTTCCC CAGGCAACTT CAGAATCCAA ATTACTTGGC    9650
ATTCCTTCAA CTGCTGATCT CTTCGTCAAT TCTGTATCTG TTGCTGCCAG    9700
CCATTCTGTT AAGACATTCA TTTCCTTTCT CATCTTACGG GACAACTTCA    9750
AGCATTTCTC CAACTGTTGC TTTCTCTCTG TTACCTTCGC ACCCAACTCA    9800
TTGTAATGCA ATTTCAAAGC TGTTACTCGT TCATCAAGCT CTTTGGGATT    9850
TTCTGTCTGC TTTTTCTGTA CAATTTGACG TCCGGTTTTA ATCACCATTT    9900
CCACTTCAGA CTTGACTTCA CTCAGGCTTT TATACAAGTT CACACAATGA    9950
CTTAGTTGTG ACTGAATTAC TTCCTGTTCA ACACTCTTGG TTTCCAATGC   10000
AGGCAAATGC ATCTTGACTT CATCTAAAAT CATCTTACTT TCCTCTAGAC   10050
GTTGTTCAAA ATTGGCTGGT TTTTGGAATA ATCGAAATTT CATGGAGACA   10100
TCTTGTAATT TTTTCTGTGC AACATCAATT TGTGAAAGAA CCCTTTGGTT   10150
```

FIGURE 12I

GGCATCCTTC CCCTGGTTAT GTTTCTTCAT TTCTTCTAAA CTTATCTCAT 10200

GACTTGTCAA ATCTGATTGG ATTTTCTGGG CTTCCTGAGG CATTTGAGCT 10250

GCATCCACCT TGTCAGTGAT ATAAGCTGCC AACTGCTTGT CAATGAATTC 10300

AAGCGACTCC TGAATTAAGT GCAAGGACTT TTCAATTTCC TGGGCAGACT 10350

GGATACTCTG TTCAAGCAAC TTTTGTTTCC TCACAGCCTC TTCATGTAGT 10400

TCCCTCCAAC GAGAATTAAA CGTCTCAAGC TCCTCATTGA TCAGTTCATC 10450

CATGACTCCT CCATCTGTAA GAGTCTGTGC CAATAGACGA ATCTGATTTG 10500

GGTTCTCCTC TGAATGATGC ATCAGATTTT CAAGAGATTC TAGCACTTCA 10550

GTGATTTCCT CAGGTCCTGC AGGAACATTT TCCATGGTTT TAAGTTTCAA 10600

TTCTACTTCA TTGAGCCACT TGTTTGCTTT CTCTAAATAT GACAATAACT 10650

CATGCCAACA TGCCCAAACT TCTTCCAAAG TTTTGCATTT TCCATTCAGC 10700

CTGGTGCACA GCCATTGGTA GTTGGTGGTC AGAGTTTCAA GTTCCTTTTT 10750

TAAGGCCTCT TGTGCTGAGG GTGGAGCGTG AGCTATTACA CTATTTACAG 10800

TCTCAGTAAG GAGTTTCACT TTAGTTTCTT TTTGTAGTGC CTCTTCTTTA 10850

GCTCTCTTCA TTTCTTCAAC AGCAGTCTGT AATTCATCTG GAGTTTTATA 10900

TTCAAAATCT CTCTCTAGAT ATTCTTCTTC AGCTTGTGTC ATCCACTCAT 10950

GCATCTCTGA TAGATCTTTT TGGAGGCTTA CGGTTTTATC CAAACCTGCC 11000

TTTAAGGCTT CCTTTCTGGT GTAGACCTGG CGGCATATGT GATCCCACTG 11050

AGTGTTAAGC TCTCTAAGTT CTGTCTCCAG TCTGGATGCA AACTCAAGTT 11100

CAGCTTCACT CTTTATCTTC TGCCCACCTT CATTAACACT ATTTAAACTG 11150

GGCTGAATTG TTTGAATATC ACCAACTAAA AGTCTGCATT GTTTGAGCTG 11200

TTTTTTCAGG ATTTCAGCAT CCCCCAGGGC AGGCCATTCC TCTTTCAGGA 11250

AAACATCAAC TTCAGCCATC CATTTCTGTA AGGTTTTTAT GTGATTCTGA 11300

AATTTTCGAA GTTTATTCAT ATGTTCTTCT AGCTTTTGGC AGCTTTCCAC 11350

CAACTGGGAG GAAAGTTTCT TCCAGTGCCC CTCAATCTCT TCAAATTCTG 11400

FIGURE 12J

```
ACAGATATTT CTGGCATATT TCTGAAGGTG CTTTCTTGGC CATCTCCTTC   11450
ACAGTGTCAC TCAGATAGTT GAAGCCATTT TGTTGCTCTT TCAAAGAACT   11500
TTGCAGAGCC TGTAATTTCC CGAGTCTCTC CTCCATTATT TCATATTCAG   11550
TAACACTAAG ATAAGGTACA GAGAGTTTGC TTTCTGACTG CTGGATCCAC   11600
GTCCTGATGC TACTCATTGT CTCCTGATAG CGCATTGGTG GTAAAGTGTC   11650
AAAAATTGTC TGTAGCTCTT TCTCTTTGGC CCTCACACCA TCAAAGATGT   11700
GGTTAAAATG ATTAGTAAAG GCCACAAAGT CTGCATCCAG AAACATTGGC   11750
CCCTGTCCCT TTTCTTTCAG TTGTAGACTC TGAATTTTTA ATTGCTCAAT   11800
TTGAGGCTGA AGAGCTGACA ATCTGTTGAC TTCATCCTTA CAAATTTTTA   11850
ACTGGCTTTT AATTGCTGTT GGCTCTGATA GGGTGGTAGA CTGGGTTTTC   11900
AACAAGTTTT CGGCAGTAGT TGTCATCTGT TCCAATTGTT GTAGCTGATT   11950
ATAAAAGGTA ATGATGTTGG TTTGATACTC TAGCCAGTTA ACTCTCTCAC   12000
TCAGCAATTG GCAGAATTCT GTCCACCGGC TGTTCAGTTG TTCTGAAGCT   12050
TGTCTGATAC TTTCAGCATT AACACCCTCA TTTGCCATCT GTTCCACCAG   12100
GGCCTGAGCT GATCTGCTGG CATCTTGCAG TTTTCTGAAC TTCTCTGCTT   12150
TTTCTCGTGC TATGGCATTG ACTTTTTCTT GCAAGTCTGA GATGTTGCCT   12200
TCTTTTCGAT AGACTGCAAA TTCAGAACTC TGTAATACAG CTTCTGAACG   12250
AGTAATCCAA CTGTGAAGTT CAGTTATATC GACATCCAAC CTTTTCCTGA   12300
GTTCAGAATC CACAGTTATC TGCCTCTTCT TTTGAGGAGG TGGTGGTGGA   12350
AGTTCCTCTT GGGCATGTTT TACCATGATT TGTTCCCTTG TGGTCACCAT   12400
AGTTACCGTT TCCATTACAG TTGTCTGTGT TAGGGATGGT TGAGTGGTGG   12450
TGACAGCCTG TGAAATTTGT GCTGAACTCT TTTCAAGTTT TTGGGTTAAA   12500
TTGTCCCAAC GTTGTGCAAA GTTTTCCATC CAGATTTCCA TCTTTTGAGT   12550
CACTGACTTA TTTTTCAGTG CCGAAAGTAG ATCTTGATTG AGTGAACTTA   12600
GTTTTTCCAT GGTTGGCTTT TTCTTTTCTA GATCTATTTT TAAAGTAGAT   12650
```

FIGURE 12K

ATTTTGTGAA GACTTGACAT CATTTCATTT TGATCTTTAA AGCCACTTGT 12700

CTGAATGTTC TTCATTGCAT CTTCTTTTTC TGAAAGCCAT GTACTAAAAA 12750

GGCACTGTTC TTCAGTAAAA TGCTGCCATT TTAGAAGAAT ATCTTGTAAA 12800

ACAATCCAGC GGTCTTCAGT CCATCTGCAG ATATTTGCCC ATCGATCTCC 12850

CAGTACCTTA AGTTGTTCTT CCAAAGCAGC TGTTGCATGA TCACCGCTGG 12900

ATTCATCAAC CACTACTACC ATGTGAGTGA GCGAGTTGAC CCTGACCTGC 12950

TCCTGTTCTA GATCTTCTTG AAGCACCTTA TGTTGTTGTA CTTGGCATTT 13000

TAGATCTTCA AGATCAGGTC CAAAGGGCTC TTCCTCCATT TTCTTAGTTC 13050

TCTCTTCAGT TTTTGTTAAC CAGTCATCTA GTTCTTTTAA TTTCTGATTC 13100

TGGAGATCCA TTAGAACTTT GTGTAATTTG CTTTGTTTTT CCATGCTAGC 13150

TACCCTGAGA CATTCCCATC TTGAATTTAG GAGATTCATT TGTTCTTGCA 13200

CTTCAGCTTC TTCATCTTCT GATAATTTCC CTTTTCCAAC TAGTTGACTT 13250

CCTAACTGTA GAACATTACC AACAAGTCCT TGATGAGATG TCAGATCCAT 13300

CATGAATCCC TCATGAGCAT GAAACTGTTC TTTCACTTCT TCAACATCAT 13350

TTGAAATCTC TCCTTGTGCT CGCAATGTAT CCTCGGCAGA AAGAAGCCAT 13400

GAAAGTACTT CTTCTAAAGC AGTTTGGTAA CTATCCAGAT TTACTTCCGT 13450

CTCCATCAAT GAACTGTCAA GTGACTTGTC TCTGGGAGCT TCCAAATGCT 13500

GTGAAGGATA GGGGCTCTGT GTGGAATCAG AGGTGGCAAC ATAAGCAGCC 13550

TGTGTGAAGG CATAACTCTT GAATCGAGGC TTAGGAGATG AAGAAGTTTG 13600

TTCATAGCCC TGTGCTAGAC TGACTGTGAT CTGTTGAGAG TAATGCATCT 13650

GGTGATGTAA TTGAAAATGT TCTTCTCTAG TTACTTTTGA AGATGTCCTG 13700

GGCAACATTT CCACTTCTTG AATGGCTTCA ATGCTCACTT GTTGTGGCAA 13750

AACTTGAAAG AGTGATGTGA TGTACATTAA GATGGACTTC TTGTCTGGAT 13800

AAGTGGTAGC AACATCTTCA GGATCAAGAA GTTTTTCTAT GCCTAACTGG 13850

CATTTTGCAA TGTTGAAGGC ATGTTCCAGT CTTTGGGTGG CTGAGTGCTG 13900

FIGURE 12L

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TGAAACCACA CTATTCCAAT CAAACAGGTC GGGCCTGTGA CTATGGATAA   13950
GAGCATTCAA AGCCAACCCG TCGGACCAGC TAGAGGTGAA GTTGATGACG   14000
TTAACCTGTG GATAATTACG TGTTGACTGT CGAACCCAGC TCAGAAGAAT   14050
CTTTTCACTG TTGGTTTGCT GCAATCCAGC CTTGATAGTT TTCATCACAT   14100
TTTTGACCTG CCAGTGGAGG ATTATATTCC AAATCAAACC AAGAGTGAGT   14150
TTATGATTTC CATCCACTAT GTCAGTGCTT CCTATATTCA CTAAATCAAC   14200
ATTATTTTTC TGTAAGACCC GCAGTGCCTT GTTGACATTG TTCAGGGCAT   14250
GAACTCTTGT AGATCCCTTT TCTTTTGGCA GTTTTTGCCC TGTAAGGCCT   14300
TCCAAGAGGT CTAGGAGGCG TTTTCCATCC TGCAGGTCAC TGAAGAGGTT   14350
GTCTATGTGT TGCTTTCCAA ACTTAGAAAA TTGTGCATTT ATCCATTTTG   14400
TGAATGTTTT CTTTTGAACA TCTTCTCTTT CATAACAGTC CTCTACTTCT   14450
TCCCACCAAA GCATTTGGAA GAAAAAGTAT ATATCAAGGC AGGGATAAAA   14500
ATCTTGGTAA AAGTTTCTCC CAGTTTTATT GCTCCAGGAG GCTTAGGTAC   14550
GATGAGAAGC CAATAAACTT CAGCAGCCTT GACAAAAAAA AAAAAAAAAA   14600
TAGCACTTCA AGTCTTCCTA TTCGTTTTTT CTATAAAGCT ATTGCCTTCA   14650
AGAGCGGAAT TCCTGCAGCC CGGGGGATCC ACTAGTTCTA GAGCGGCCGC   14700
GGGTACAATT CCGCAGCTTT TAGAGCAGAA GTAACACTTC CGTACAGGCC   14750
TAGAAGTAAA GGCAACATCC ACTGAGGAGC AGTTCTTTGA TTTGCACCAC   14800
CACCGGATCC GGGACCTGAA ATAAAAGACA AAAAGACTAA ACTTACCAGT   14850
TAACTTTCTG GTTTTTCAGT TCCTCGAGTA CCGGATCCTC TAGAGTCCGG   14900
AGGCTGGATC GGTCCCGGTG TCTTCTATGG AGGTCAAAAC AGCGTGGATG   14950
GCGTCTCCAG GCGATCTGAC GGTTCACTAA ACGAGCTCTG CTTATATAGA   15000
CCTCCCACCG TACACGCCTA CCGCCCATTT GCGTCAATGG GGCGGAGTTG   15050
TTACGACATT TTGGAAAGTC CCGTTGATTT TGGTGCCAAA ACAAACTCCC   15100
ATTGACGTCA ATGGGGTGGA GACTTGGAAA TCCCCGTGAG TCAAACCGCT   15150
ATCCACGCCC ATTGATGTAC TGCCAAAACC GCATCACCAT GGTAATAGCG   15200
```

FIGURE 12M

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ATGACTAATA CGTAGATGTA CTGCCAAGTA GGAAAGTCCC ATAAGGTCAT   15250
GTACTGGGCA TAATGCCAGG CGGGCCATTT ACCGTCATTG ACGTCAATAG   15300
GGGGCGTACT TGGCATATGA TACACTTGAT GTACTGCCAA GTGGGCAGTT   15350
TACCGTAAAT ACTCCACCCA TTGACGTCAA TGGAAAGTCC CTATTGGCGT   15400
TACTATGGGA ACATACGTCA TTATTGACGT CAATGGGCGG GGGTCGTTGG   15450
GCGGTCAGCC AGGCGGGCCA TTTACCGTAA GTTATGTAAC GACCTGCAGG   15500
TCGACTCTAG AGGATCTCCC TAGACAAATA TTACGCGCTA TGAGTAACAC   15550
AAAATTATTC AGATTTCACT TCCTCTTATT CAGTTTTCCC GCGAAAATGG   15600
CCAAATCTTA CTCGGTTACG CCCAAATTTA CTACAACATC CGCCTAAAAC   15650
CGCGCGAAAA TTGTCACTTC CTGTGTACAC CGGCGCACAC CAAAAACGTC   15700
ACTTTTGCCA CATCCGTCGC TTACATGTGT TCCGCCACAC TTGCAACATC   15750
ACACTTCCGC CACACTACTA CGTCACCCGC CCCGTTCCCA CGCCCCGCGC   15800
CACGTCACAA ACTCCACCCC CTCATTATCA TATTGGCTTC AATCCAAAAT   15850
AAGGTATATT ATTGATGATG CTAGCGGGGC CCTATATATG GATCCAATTG   15900
CAATGATCAT CATGACAGAT CTGCGCGCGA TCGATATCAG CGCTTTAAAT   15950
TTGCGCATGC TAGCTATAGT TCTAGAGGTA CCGGTTGTTA ACGTTAGCCG   16000
GCTACGTATA CTCCGGAATA TTAATAGGCC TAGGATGCAT ATGGCGGCCG   16050
GCCGCCTGCA GCTGGCGCCA TCGATACGCG TACGTCGCGA CCGCGGACAT   16100
GTACAGAGCT CGAGAAGTAC TAGTGGCCAC GTGGGCCGTG CACCTTAAGC   16150
TTGGCACTGG CCGTCGTTTT ACAACGTCGT GACTGGGAAA ACCCTGGCGT   16200
TACCCAACTT AATCGCCTTG CAGCACATCC CCCTTTCGCC AGCTGGCGTA   16250
ATAGCGAAGA GGCCCGCACC GATCGCCCTT CCCAACAGTT GCGCAGCCTG   16300
AATGGCGAAT GGCGCCTGAT GCGGTATTTT CTCCTTACGC ATCTGTGCGG   16350
TATTTCACAC CGCATACGTC AAAGCAACCA TAGTACGCGC CCTGTAGCGG   16400
CGCATTAAGC GCGGCGGGTG TGGTGGTTAC GCGCAGCGTG ACCGCTACAC   16450
```

FIGURE 12N

```
TTGCCAGCGC CCTAGCGCCC GCTCCTTTCG CTTTCTTCCC TTCCTTTCTC   16500
GCCACGTTCG CCGGCTTTCC CCGTCAAGCT CTAAATCGGG GGCTCCCTTT   16550
AGGGTTCCGA TTTAGTGCTT TACGGCACCT CGACCCCAAA AAACTTGATT   16600
TGGGTGATGG TTCACGTAGT GGGCCATCGC CCTGATAGAC GGTTTTTCGC   16650
CCTTTGACGT TGGAGTCCAC GTTCTTTAAT AGTGGACTCT TGTTCCAAAC   16700
TGGAACAACA CTCAACCCTA TCTCGGGCTA TTCTTTTGAT TTATAAGGGA   16750
TTTTGCCGAT TTCGGCCTAT TGGTTAAAAA ATGAGCTGAT TTAACAAAAA   16800
TTTAACGCGA ATTTTAACAA AATATTAACG TTTACAATTT TATGGTGCAC   16850
TCTCAGTACA ATCTGCTCTG ATGCCGCATA GTTAAGCCAG CCCCGACACC   16900
CGCCAACACC CGCTGACGCG CCCTGACGGG CTTGTCTGCT CCCGGCATCC   16950
GCTTACAGAC AAGCTGTGAC CGTCTCCGGG AGCTGCATGT GTCAGAGGTT   17000
TTCACCGTCA TCACCGAAAC GCGCGAGACG AAAGGGCCTC GTGATACGCC   17050
TATTTTTATA GGTTAATGTC ATGATAATAA TGGTTTCTTA GACGTCAGGT   17100
GGCACTTTTC GGGGAAATGT GCGCGGAACC CCTATTTGTT TATTTTTCTA   17150
AATACATTCA AATATGTATC CGCTCATGAG ACAATAACCC TGATAAATGC   17200
TTCAATAATA TTGAAAAAGG AAGAGTATGA GTATTCAACA TTTCCGTGTC   17250
GCCCTTATTC CCTTTTTTGC GGCATTTTGC CTTCCTGTTT TTGCTCACCC   17300
AGAAACGCTG GTGAAAGTAA AAGATGCTGA AGATCAGTTG GGTGCACGAG   17350
TGGGTTACAT CGAACTGGAT CTCAACAGCG GTAAGATCCT TGAGAGTTTT   17400
CGCCCCGAAG AACGTTTTCC AATGATGAGC ACTTTTAAAG TTCTGCTATG   17450
TGGCGCGGTA TTATCCCGTA TTGACGCCGG GCAAGAGCAA CTCGGTCGCC   17500
GCATACACTA TTCTCAGAAT GACTTGGTTG AGTACTCACC AGTCACAGAA   17550
AAGCATCTTA CGGATGGCAT GACAGTAAGA GAATTATGCA GTGCTGCCAT   17600
AACCATGAGT GATAACACTG CGGCCAACTT ACTTCTGACA ACGATCGGAG   17650
GACCGAAGGA GCTAACCGCT TTTTTGCACA ACATGGGGGA TCATGTAACT   17700
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FIGURE 120

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CGCCTTGATC GTTGGGAACC GGAGCTGAAT GAAGCCATAC CAAACGACGA   17750
GCGTGACACC ACGATGCCTG TAGCAATGGC AACAACGTTG CGCAAACTAT   17800
TAACTGGCGA ACTACTTACT CTAGCTTCCC GGCAACAATT AATAGACTGG   17850
ATGGAGGCGG ATAAAGTTGC AGGACCACTT CTGCGCTCGG CCCTTCCGGC   17900
TGGCTGGTTT ATTGCTGATA AATCTGGAGC CGGTGAGCGT GGGTCTCGCG   17950
GTATCATTGC AGCACTGGGG CCAGATGGTA AGCCCTCCCG TATCGTAGTT   18000
ATCTACACGA CGGGGAGTCA GGCAACTATG GATGAACGAA ATAGACAGAT   18050
CGCTGAGATA GGTGCCTCAC TGATTAAGCA TTGGTAACTG TCAGACCAAG   18100
TTTACTCATA TATACTTTAG ATTGATTTAA AACTTCATTT TTAATTTAAA   18150
AGGATCTAGG TGAAGATCCT TTTTGATAAT CTCATGACCA AAATCCCTTA   18200
ACGTGAGTTT TCGTTCCACT GAGCGTCAGA CCCCGTAGAA AAGATCAAAG   18250
GATCTTCTTG AGATCCTTTT TTTCTGCGCG TAATCTGCTG CTTGCAAACA   18300
AAAAAACCAC CGCTACCAGC GGTGGTTTGT TTGCCGGATC AAGAGCTACC   18350
AACTCTTTTT CCGAAGGTAA CTGGCTTCAG CAGAGCGCAG ATACCAAATA   18400
CTGTTCTTCT AGTGTAGCCG TAGTTAGGCC ACCACTTCAA GAACTCTGTA   18450
GCACCGCCTA CATACCTCGC TCTGCTAATC CTGTTACCAG TGGCTGCTGC   18500
CAGTGGCGAT AAGTCGTGTC TTACCGGGTT GGACTCAAGA CGATAGTTAC   18550
CGGATAAGGC GCAGCGGTCG GGCTGAACGG GGGGTTCGTG CACACAGCCC   18600
AGCTTGGAGC GAACGACCTA CACCGAACTG AGATACCTAC AGCGTGAGCT   18650
ATGAGAAAGC GCCACGCTTC CCGAAGGGAG AAAGGCGGAC AGGTATCCGG   18700
TAAGCGGCAG GGTCGGAACA GGAGAGCGCA CGAGGGAGCT TCCAGGGGGA   18750
AACGCCTGGT ATCTTTATAG TCCTGTCGGG TTTCGCCACC TCTGACTTGA   18800
GCGTCGATTT TTGTGATGCT CGTCAGGGGG GCGGAGCCTA TGGAAAAACG   18850
CCAGCAACGC GGCCTTTTTA CGGTTCCTGG CCTTTTGCTG GCCTTTTGCT   18900
CACATGTTCT TTCCTGCGTT ATCCCCTGAT TCTGTGGATA ACCGTATTAC   18950
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FIGURE 12P

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CGCCTTTGAG TGAGCTGATA CCGCTCGCCG CAGCCGAACG ACCGAGCGCA   19000
GCGAGTCAGT GAGCGAGGAA GCGGAAGAGC GCCCAATACG CAAACCGCCT   19050
CTCCCCGCGC GTTGGCCGAT TCATTAATGC AGCTGGCACG ACAGGTTTCC   19100
CGACTGGAAA GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA   19150
CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC TCGTATGTTG   19200
TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCTATGACCA   19250
TGATTACGAA TTCGAATGGC CATGGGACGT CGACCTGAGG TAATTATAAC   19300
CCGGGCC                                                 19307
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